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BASIDIOBOLUS AND CERCOSPORA FROM HUMAN INFECTIONS

C. W. EMMONS,[1] LIE-KIAN-JOE,[2] NJO-INJO TJOEI ENG,[2] A. POHAN,[3] S. KERTOPATI [4] AND A. VAN DER MEULEN

(WITH 4 FIGURES)

An important development of medical mycology has been its closer integration with the broad fields of mycology and plant pathology. Fungi which are well known as agents of fatal disease in man are a part of the common saprophytic flora of suitable environments, and fungi which are not ordinarily pathogenic for man can, under unusual circumstances, cause human disease.

The first of these conditions has been firmly established in recent years by several investigators and evidence on the role of soil and organic debris as habitats of infectious agents of mycoses has been reviewed recently (**1**, **7**). Evidence for pathogenicity of fungi only rarely found in human disease is more equivocal because it is based in some instances on case reports in which an etiologic relationship was not clearly established and even in cases where the evidence for etiologic relationship is acceptable, these instances are so sporadic as to make any generalizations difficult. Certain fungi such as *Absidia corymbifera* and *Aspergillus fumigatus* might be said to fall between those fungi best

[1] From the U. S. Department of Health, Education and Welfare, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland.

[2] Departments of Parasitology and General Pathology, University of Indonesia, Djakarta.

[3] Department of Surgery, University of Indonesia, Djakarta.

[4] Department of Dermatology, University of Indonesia, Djakarta.

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known to the general mycologist and plant pathologist and those more familiar to the medical mycologist. They are well known and frequently present in man's environment, yet they can and do cause fatal disease in man often enough so that they are familiar to the mycologist working exclusively with pathological material from man and animals.

We have recently reported elsewhere (**9**) clinical and mycological details concerning two cases of extensive subcutaneous lesions caused by *Basidiobolus ranarum* and a report on one case of cutaneous infection caused by *Cercospora apii* is in manuscript.[5] The three cases occurred in Indonesia. There are earlier reports of two cases of infections, quite different clinically and histologically from ours, attributed to *Basidiobolus* (**2**, **3**, **10**). Except for these our cases appear to be unique. Our cases are reviewed here in order to call the attention of mycologists to the pathogenic potentialities of these two fungi.

The first patient was an Indonesian boy, age 4 years, who was brought to the clinic complaining of a subcutaneous lesion extending over the left chest anteriorly and into the left axilla. The lesion consisted of a firm disk reaching 1.5 cm in thickness, lying between the skin and the fascia and not attached, so that it was freely moveable over the muscles. The overlying skin was bluish-red with a few areas of softening, but was otherwise not involved. The general condition of the patient was good and he complained only of the swelling, which was not painful. The patient's parents reported that the lesion began as a small subcutaneous nodule about 6 months before admission to the hospital. No history of a pre-existing insect bite or thorn injury could be obtained. The lesion had gradually increased in size until the time of admission to the hospital. Thereafter, although no specific therapy was given, the lesion decreased in size and completely disappeared 3 months after the patient was first seen at the hospital.

At the time of hospitalization a biopsy was taken from the lesion. Sections of this showed the presence of granulomatous tissue in which fungus hyphae were readily demonstrated (FIG. 1). These hyphae are non-pigmented and have few septa. Diameters vary between 8 and 12 μ and occasional cells reach a diameter of 22 μ. The fungus has the general aspect usually associated with growth of a phycomycete in animal tissue.

Since all of the first biopsy tissue had been fixed for sections a second biopsy was taken. Sections of a portion of this tissue showed similar histologic changes and the same fungus. A part of the biopsy was

[5] Lie-Kian-Joe; Tjoei Eng Njo-Injo; Sartono Kertopati and C. W. Emmons. A new verrucous mycosis caused by *Cercospora apii*. In ms.

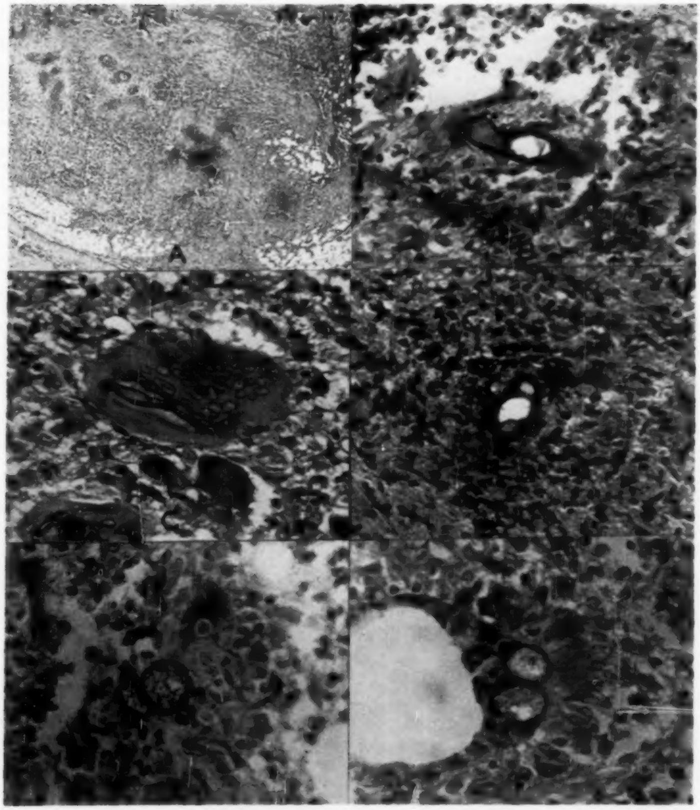

FIG. 1. *Basidiobolus ranarum* in subcutaneous lesion. A. Granulomatous lesion, × 15. B. Hyphal fragment. C. Hyphal fragment in giant cell. D–F. Swollen spherical fungus cells. B–F × 560. Periodic acid-Schiff stain with hematoxylon counter stain.

finely divided and planted on culture media and 55 colonies of a rapidly growing fungus were isolated. From a third biopsy 10 colonies of the same fungus were obtained.

The fungus produces on Sabouraud's agar (1% neopeptone, 2% glucose) incubated at 30° C, a flat colony reaching a diameter of 7 cm in 4 days. Growth at 37° C is slow. The colony is essentially a thin film of gray to pale yellow mycelium growing superficially on the agar. Its surface is radially striated and folded and after a few days is covered

with a white bloom produced by very short aerial hyphae. Microscopic examination reveals the presence of septate vegetative hyphae 8–20 μ in diameter and of many chlamydospores, conidia and zygospores. The zygospores are spherical and 40–50 μ in diameter. The exterior surface

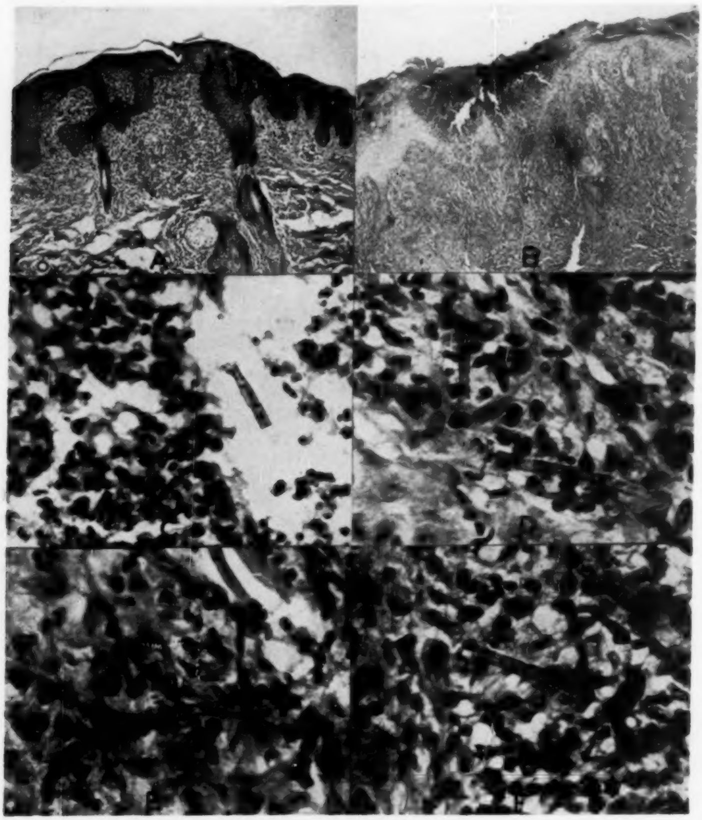

FIG. 2. *Cercospora apii* in subcutaneous lesions. A. Small subcutaneous granuloma, $\times$ 52. B. Ulcerated lesion, $\times$ 16. C–F. Hyphal fragments in lesion, $\times$ 690. Periodic-Schiff-hematoxylon stain.

of the thick wall is roughened by pits and warty excresences. The conspicuous remnants of the copulation tubes and the characteristics of the zygospores readily permit identification of the fungus as a *Basidiobolus* (FIG. 3, a–b).

A review of the literature on *Basidiobolus* indicated that *B. lacertae* and some of the other species names proposed are probably synonyms of the type species, *B. ranarum* Eidam 1887. Drechsler has recently reviewed the status of some of these names and has described two new species (**5**, **6**). The fungus isolated from this patient resembled *B. ranarum* in appearance and dimensions, although it is variable and has undergone some changes during the months it has been subcultured in the laboratory. In order to make an actual comparison, *B. ranarum* was isolated from a colony of *Bufo marinus* held in the laboratory at the National Institutes of Health and from *B. melanosticus* and *Rana cancrivora* in Indonesia. There are differences in the strains isolated from these sources, but they are minor and do not appear to justify erecting a new name for the strain isolated from a human lesion. Until a critical monographic study of *Basidiobolus* from different hosts and various geographic areas more narrowly delimits its species, we prefer to identify this fungus with *B. ranarum*.

Attempts to reproduce the disease in animals have not as yet been successful. Injection of a suspension of spores into an experimental animal produces a very small lesion in which the zygospores and chlamydospores of the inoculum can be observed, but there is no apparent growth of the fungus nor spread of the lesion. Such failure to produce an experimental lesion is a common experience with fungi.

The second patient was an Indonesian boy, age 8 years, with a similar subcutaneous lesion which began as a small nodule on the thorax 2 years before admission. The lesion increased in size to cover extensive areas of the thorax, both upper arms, the axillae, the abdomen, flanks and buttocks. In spite of the extensive areas involved the patient's general condition was good.

Two biopsy specimens taken at the time of admission were examined by a pathologist, but the nature of the lesion was not recognized. Two years later these sections were reviewed by one of us and fungus hyphae resembling those seen in the first case were found. The patient was recalled to the hospital. At this time, about 4 years after the lesion first appeared, the patient had almost recovered. The only remaining lesion was a small swelling 10 cm in diameter. A biopsy from this area showed only a healing lesion in which the fungus could not be found.

Although no fungus was isolated in culture the diagnosis of an infection produced by *B. ranarum* was made in the second case because the clinical course, the histopathology and the appearance of the fungus in the tissues were similar in the two cases.

Overeem (**9**) in 1925 reported isolation of *B. ranarum* from fungus

granules found in draining sinus tracts in a disease of a horse. Casagrandi (**2**, **3**) in 1931 reported observing zygospores of *Basidiobolus* on the surface of a gastric ulcer. This report is not convincing, and the appearance of the fungus in tissue in our cases is quite unlike that in either of the cited reports.

The second unusual mycotic pathogen of man, *Cercospora apii*, was isolated from verrucous lesions on the face of an Indonesian boy, age 12 years. Almost the entire face, including the lips and eyelids, and both ears, was covered with discrete and confluent, bluish-red, flat to cauliflower-like areas of induration. The mucosa of both nasal cavities was also affected and there was perforation of the septum (FIG. 4). The

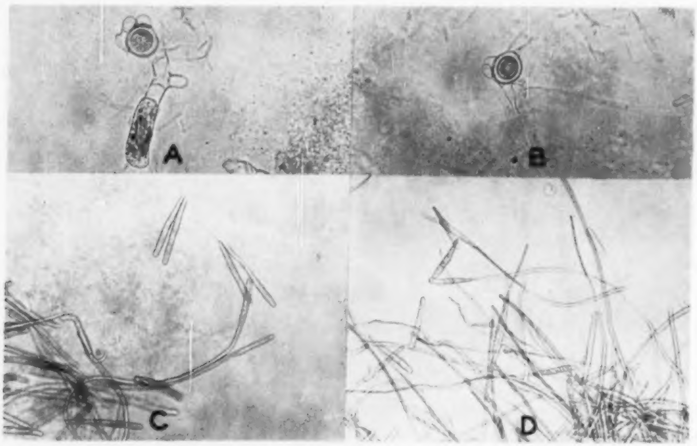

FIG. 3. *Basidiobolus ranarum* (A–B) and *Cercospora apii* (C–D) spores from culture, × 144.

patient was otherwise well, without fever or lymph node enlargement. During the further course of the disease a papule developed on the left thorax under the clavicle and another on the dorsal side of the right thigh.

The parents stated that the lesions began many years before with a small nodule on the left cheek, but they were unable to relate it to any thorn injury or insect bite, nor could they remember whether poultices prepared from leaves or other plant materials had been used in the treatment.

Eight biopsies were taken at different times and from different areas

of the lesions on the face. Fungus hyphae were found in all. The hyphae have brown walls, visible in an unstained section, are 4–8 μ in diameter and have frequent septa. They are present in all layers of the skin, including the corium. The hyphae are remarkably uniform in diameter and do not present the contorted abnormal forms often associated with the growth of a fungus in mammalian tissue. Hypertrophy and hyperplasia are apparent in the epithelium and granulomatous reactions occur in the corium (FIG. 2). More detailed descriptions of the histopathology are given in the case report.[5]

Attempts to isolate the fungus in culture from four biopsies yielded some 150 colonies of *Cercospora*. The fungus does not grow at 37° C. At room temperature and at 30° C it grows slowly and on cornmeal agar after 10 days the colony is dome-shaped with a height of 4–5 mm and a diameter of 15 mm. The texture appears plush-like with a tough, compact base and very short aerial hyphae. The color is olivaceous. On Sabouraud's agar the colony presents a somewhat higher dome and is darker in color.

Sporulation occurs on both media and the conidiophores and conidia vary greatly in shape and dimensions (FIG. 3, c–d). The conidiophores are brown, poorly differentiated hyphal branches. Many conidiophores bear a slight resemblance to those of *Helminthosporium*, with barely discernible lateral and terminal scars from which conidia are detached very early and easily.

The conidia are pale brown, clavate to acicular, with truncated (usually convex) base and a rounded or greatly elongated whip-like tip. When a preparation for microscopic examination is made, nearly all the conidia become detached. These immature conidia may be unicellular, but mature conidia have three to several septa. The spores are never muriform. The conidia from this strain were 4–6 × 26–120 μ in size. It is generally recognized that both shape and size of *Cercospora* conidia are so variable as to be nearly worthless in determination of species.

Since species of *Cercospora* have been based almost entirely upon the species of host plant, the character of the lesion and the geographical area from which a collection was made, there are few criteria to guide one in the identification of a strain isolated from a saprophytic or human source. Chupp's monograph (**4**) lists nearly 2400 species of host plants and about 3800 species of *Cercospora* of which he accepts some 1800 as valid species. This monograph provides no way by which a mycologist with a pure culture of *Cercospora* can identify it with any one of the 1800 species.

Only a few mycological studies throw light on this taxonomic morass. Johnson and Valleau (**8**) attempted cross inoculations with pure cultures of Cercosporas from 28 host species representing 16 plant families. They produced "frog-eye" on Burley tobacco with fungi isolated from 16 host species representing 11 plant families. They conclusively disproved the previously assumed host specificity of *Cercospora* and listed 45 species names as synonyms of *Cercospora apii* Fresenius 1863. While reduction of the present 3000 species of *Cercospora* to a few

FIG. 4. Lesions in which brown hyphae are easily demonstrated and from which 150 colonies of *Cercospora apii* were isolated. Photograph made about one year after patient's admission to hospital.

valid names may be impracticable of accomplishment, this and similar studies (**8, 11, 12**) show conclusively that the present species concepts in *Cercospora* are unrealistic.

Cercospora apii is known throughout the world where celery is grown. The synonymy of this species proposed by Johnson and Valleau includes *C. beticola* from sugar beet and *C. nicotiana* from tobacco. According to their concept *C. apii* is a species of wide geographic distribution and host range. The fungus isolated from our patient is com-

patible in morphology and spore dimensions with that species. Instead of adding another specific name to the poorly defined 3000 now in the literature, we therefore have identified the fungus as *C. apii.*

This identification made it necessary to investigate the phytopathogenicity of the fungus. Accordingly young or seedling plants of 9 species were taken from a coldframe and garden, potted and brought to the laboratory. Spore suspensions of the fungus isolated from human lesions were prepared from an 18-day culture on cornmeal agar and applied to the leaves of plants which were then placed in moist chambers. Tween-80 was used to wet leaves in a few cases. After one week leaf spots in which hyphae and conidia of *Cercospora* were abundantly present were observed on lettuce, tomato and potato. *Cercospora,* indistinguishable from the strain used for experimental inoculation, was isolated from lettuce, but cultures from the other plants were overgrown with other fungi. The conidia found on the leaf spots included more long-acicular forms than were observed in culture. However, as previously pointed out, it is generally recognized that spore dimensions vary greatly, and the size of conidia from the leaves fell within the range observed in culture. The possibility that wild *Cercospora* spores were already present on the leaves of the experimental plants and gave rise to the leaf spots observed cannot be excluded. The observations add some support, however, to the conclusion that the fungus isolated from the patient is *Cercospora apii.*

Experimental production of lesions in animals, using spore suspensions of this fungus, has not as yet been successful although the fungus can be recovered in culture from peritoneal surfaces of mice inoculated some weeks previously.

SUMMARY

Two cases of subcutaneous infection in children in Indonesia in which fungus hyphae of wide dimensions and with few septa were observed have been reported. From one of these patients 65 colonies of *Basidiobolus ranarum* were isolated. The second patient had almost recovered from the infection before a mycological study was made and no fungus was isolated. The lesions were extensive and persisted 9 months in one case and 4 years in the other. Although no specific therapy was used the patients recovered.

From a third Indonesian patient with cutaneous and subcutaneous lesions involving most of the face, ears and nasal mucosa, 150 colonies of *Cercospora apii* were isolated. In sections of the skin the fungus is easily observed in the form of brown hyphae with frequent septation.

The infection has persisted for many years and attempts at specific therapy have failed.

Leaf spots were produced experimentally on lettuce, potato and tomato with the strain of *C. apii* isolated from the patient. Persistent and progressive lesions have not been produced in attempted experimental infections of animals with either *Basidiobolus ranarum* or *Cercospora apii*.

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EFFECT OF TEMPERATURE AND NUTRITION UPON MACROCONIDIAL FORMATION OF MICROSPORUM AUDOUINI [1]

Elizabeth L. Hazen

(with 1 figure)

Investigations (**1–5**, **7**, **8**, **12**, **14–17**) of the effect of nutrition on growth and morphology of the dermatophytes have been conducted for some time in this department. Of chief interest to this author has been the study of the factors involved in macroconidial formation by *Microsporum audouini*. The definitive identification of this species alone of the genus *Microsporum* is a problem in the diagnostic laboratory because of the difficulty of inducing the formation of macroconidia that are essential for species differentiation.

In an earlier study (**7**) it was found that additions of yeast extract (5 mg per milliliter) to honey agar caused a marked increase in vegetative growth and macroconidial formation. Furthermore, it was observed that moderate mycelial growth was supported by the honey agar alone, but few macroconidia were produced, and in many cultures none could be found. Additions of pyridoxine (0.5 to 1 μg per milliliter) caused no increase in vegetative growth but did induce some increase in numbers of macroconidia. Additions of thiamine (0.5 to 1 μg per milliliter of medium) or thiamine and pyridoxine caused no increase in macrospore formation. In a subsequent study (**8**) in which a variety of nutrients were incorporated in rice infusion agar and a chemically defined agar medium, it appeared that a source of nitrogen suitable for the particular strain was necessary for macrospore formation. No factor was found which induced abundant macrospore formation in all strains of *M. audouini*, the abundance of the spores apparently depending not only upon the medium used but also upon some property of the strain itself.

Following the early report (**7**), honey agar plus yeast extract was used routinely with success in the diagnostic laboratory for stimulation

[1] This work was done in the Department of Dermatology, College of Physicians and Surgeons, Columbia University, in association with Dr. Rhoda Benham, to whom I wish to express thanks for advice and suggestions throughout the study.

of macroconidial formation of isolates of *M. audouini* recovered from the scalps of children treated at the Vanderbilt Clinic for tinea capitis. Some years later, however, repeated failures to demonstrate the macrospores in fresh isolates, otherwise typical of this species, on honey agar plus yeast extract were encountered.

Efforts to find an explanation for the recent failures of fresh isolates of *M. audouini* to produce macroconidia revealed no differences in the preparation of the medium nor in the manner in which it was used. A notable change, however, had occurred in the cultural environment. The location of the laboratory had changed. The temperature in the room used for the later studies was considerably higher at all seasons than in the room where the earlier work was done, although unfortunately no records are available of the temperature of the room in which the early work had been carried out. The minimum and maximum temperatures of the present laboratory room recorded from February to August at 9 A.M. and 5 P.M. ranged generally between 29 and 32° C, the extreme readings being 27 and 34.5° C.

In many laboratories it is common practice to incubate fungus cultures at uncontrolled room temperature which is subject to great variation. Whether or not the incubation temperature accounts for some of the difficulty encountered in the demonstration of macroconidial formation of *M. audouini* seemed worthy of investigation.

Sabouraud (**13**) stated that at incubation higher than 25° C, *M. audouini* shows deterioration. Kadisch (**10**), in 1930, employing room temperature, 27 and 37° C, reported that growth of dermatophytes was best at 27° C. Giblett and Henry (**6**) in their physiologic studies of the three species of *Microsporum* showed that the growth of five isolates of *M. audouini*, as determined by colony size, was maximum throughout the range of 25 to 30° C; that a marked reduction in growth occurred at 35 and 37° C, and at 38° C growth was almost completely inhibited.

Skinner, Emmons, and Tsuchiya (**18a**) state that in general 30° C falls near the optimum for most common pathogenic and saprophytic fungi. Jones (**9**) in a study of factors affecting the production of resistant sporangia of *Allomyces arbuscula* concluded that temperature is an important controlling factor in the production of resistant sporangia in his cultures of this fungus grown on maltose-peptone agar, and that within certain limits the total amount of heat to which cultures are subjected is more important than the maximum, minimum, or degrees of fluctuation of temperature. Many investigators have found that there is an optimum temperature for sporulation as well as for growth and that the two optima may be different (Lilly and Barnett, **11**). Our

experience with *M. audouini* is in accord with these observations. Henry and Andersen (quoted by Lilly and Barnett, **11**) found that a temperature slightly above the optimum has a much greater effect upon the number of spores produced by *Piricularia oryzae* than a slight decrease in temperature below the optimum. We are unaware of any previous study of the influence of temperature upon macroconidial formation of *M. audouini.*

Therefore in the further study of the nutritional factors involved in macrospore formation of this fungus, the influence of temperature upon development of these spores was also investigated. Two temperature ranges were selected for study: that of the laboratory room which fluctuated for the most part between 29 and 32° C and that of an air-conditioned room maintained at 25 to 27° C. These temperature ranges were chosen since the higher range was that at which failure to obtain macroconidia occurred and the lower conformed more with the apparent requirements of this species. (No attempt was made at this time to determine the optimum temperature for macrospore formation.) Yeast extract, incorporated in honey agar (**7**), was restudied for its macroconidia-enhancing property under the two selected temperatures of incubation. In addition, mixtures of the vitamins and/or amino acids of yeast extract as well as yeast extract itself were studied by incorporating them in a chemically defined agar medium, basal medium A (**8**) without asparagin, and incubating at the two ranges of temperature. (Previous tests had shown that *M. audouini* grew well on these latter media.) The results of this study are reported.

MATERIALS AND METHODS

Fresh isolates obtained from the scalps of children treated at the Vanderbilt Clinic for tinea capitis were employed. Their macroscopic growth feature and pigment formation on Sabouraud's glucose agar were characteristic of *M. audouini* but owing to the absence of macroconidia, they had not been identified definitely on Saubouraud's honey or glucose agar or on these media to which yeast extract had been added. The isolates were maintained on Saubouraud's glucose agar and were transferred usually at monthly intervals.

The yeast extract (a dehydrated Difco product) was prepared in a 10% solution in distilled water, sterilized by passage through a Seitz filter, and added to the medium in a concentration of 10 mg per milliliter of medium. The organic components, B vitamins and amino acids, in yeast extract, according to an analysis kindly furnished by the Difco Laboratories, were used.

Fresh sterile mixtures of the crystalline vitamins and amino acids (with the exception of tyrosine) in distilled water were made separately. The final concentrations of the substances in the basal medium were calculated to supply the same concentrations as that provided by 10 mg of yeast extract per milliliter of medium. Tyrosine in the crystalline form was added directly to the medium.

The amounts of the substances per milliliter of medium were as follows:

Vitamins: biotin, 0.014 μg; nicotinic acid, 2.8 μg; pyridoxine, 0.2 μg; riboflavin, 0.19 μg; thiamine, 0.032 μg.

Amino acids: L^+ arginine, 0.08 mg; DL aspartic acid, 0.51 mg; L glutamic acid, 0.65 mg; glycine, 0.24 mg; L histidine, 0.094 mg; DL isoleucine, 0.3 mg; DL leucine, 0.36 mg; L lysine, 0.4 mg; DL methionine, 0.08 mg; DL phenylalanine, 0.22 mg; DL threonine, 0.34 mg; L tryptophane, 0.09 mg; L tyrosine, 0.06 mg; DL valine, 0.34 mg.

Ten isolates (Nos. *36–45*) were studied on the various media in slide culture preparations (**18b**) for macroconidial formation with incubation at the two temperature ranges, 25 to 27° C and 29 to 32° C. The inocula for the slide culture preparations of honey agar and honey agar plus yeast extract were taken directly from the growth on Saubouraud's glucose agar slants incubated, respectively, at the two temperatures. For the chemically defined media, the mycelium was washed five times in relatively large amounts of distilled water and used as the inoculum.

Bits of the mycelium were streaked in two rows on duplicate slides containing approximately 0.32 ml of the medium. One set was incubated for two weeks and the other for four weeks. At the end of the incubation period, the preparations were dehydrated, mounted, and examined for macrocinidia. The effects of temperature and nutrients upon macroconidial formation were evaluated on the basis of the largest number of macrocinidia found in one of the two slide culture preparations.

RESULTS

The results of the experiments are summarized in TABLES I and II.

TABLE I—Honey agar: Macroconidia typical in morphology of *M. audouini* were found in small numbers in five of 10 isolates on the honey agar incubated at 25 to 27° C, while none were present in any of the 10 isolates on the same medium incubated at 29 to 32° C.

Macroconidia of typical morphology were present in nine of 10 isolates on the honey agar plus yeast extract at 25 to 27° C. In contrast, macroconidia were found in only one of the 10 isolates on the

TABLE I

EFFECT OF TEMPERATURE UPON MACROCONIDIAL FORMATION* OF M. AUDOUINI ON HONEY AGAR AND HONEY AGAR PLUS YEAST EXTRACT

Culture No.	25–27° C		29–32° C	
	Honey agar	Honey agar plus yeast extract (10 mg/ml)	Honey agar	Honey agar plus yeast extract (10 mg/ml)
	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks
36	+	++	0	0
37	0	0	0	0
38	0	++	0	0
39	0	++++	0	0
40	0	++	0	0
41	0	++++	0	0
42	+	++++	0	0
43	+	+	0	0
44	+	++++	0	++++
45	+	++	0	0

* No spores = 0; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++.

TABLE II

EFFECT OF TEMPERATURE UPON MACROCONIDIAL FORMATION* OF M. AUDOUINI ON BASAL MEDIUM WITH YEAST EXTRACT OR ORGANIC COMPONENTS OF YEAST EXTRACTS

Culture No.	25–27° C			29–32° C		
	Basal medium plus yeast extract (10 mg/ml)	Basal medium plus mixture of B vitamin and amino acids†	Basal medium plus amino acids†	Basal medium plus yeast extract (10 mg/ml)	Basal medium plus mixture of B vitamin and amino acids†	Basal medium plus amino acids†
	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks	Macroconidia 2–4 weeks
36	++++	0	0	0	0	....
37	+	0	++	+	+	contaminated
38	+	0	0	0	0	0
39	+	0	0	+	0	....
40	++	0	0	0	0	....
41	++	++	0	0	0	....
42	++++	++	+	++++	0	0
43	+	0	0	+	0	....
44	++++	+	+	++++	++	0
45	0	0	0	0	0	....

* No spores = 0; 1–5 spores = +; 6–10 spores = ++; more than 10 spores = ++++; test not performed = ...

† Concentration of substances per milliliter of medium equal to amount present in 10 mg of yeast extract.

same medium at 29 to 32° C. This isolate produced in abundance spores of typical morphology.

These results indicate that temperature is a controlling factor in the macroconidial development of *M. audouini* on honey agar and honey agar plus yeast extract. For maximum development of the spores some unknown factor or factors in yeast extract were found to be essential.

TABLE II—Basal medium: Macroconidia typical in morphology of *M. audouini* (FIG. 1) were present in nine of 10 isolates on the basal medium plus yeast extract at a temperature of 25 to 27° C. On the same medium at 29 to 32° C macrospores were found in only five of 10 isolates.

Macroconidia were present in only three of 10 isolates on the basal medium plus the combination of amino acids and B vitamins at 25 to 27° C and in only two isolates at 29 to 32° C.

Macrospores were found in three of 10 isolates on the basal medium plus the amino acids at 25 to 27° C and none in the three isolates tested at 29 to 32° C.

These results indicate that the temperature of incubation is a controlling factor in macroconidial formation of *M. audouini* on basal medium plus yeast extract. The crystalline vitamins and/or amino acids, under the conditions tested, were inadequate as a substitute for yeast extract for macroconidial production.

DISCUSSION

This study indicates that incubation temperature was the controlling factor in the macroconidial development of *M. audouini* on honey agar or basal medium plus yeast extract. The addition of yeast extract to honey agar with incubation at 25 to 27° enhanced to a marked degree macroconidial formation, nine of the isolates forming the macrospores, while on the identical medium with incubation at a temperature which fluctuated for the most part between 29 and 32° C only one developed the spores. The assumption seems justified that incubation temperature and not inadequate medium was responsible for the recent failures in the diagnostic laboratory to demonstrate macroconidia in fresh strains of *M. audouini* on honey agar plus yeast extract. The fact that on honey agar five of the 10 isolates formed a few macroconidia at 25 to 27° C whereas none formed the spores at the higher temperature adds further proof to this assumption.

These results suggest that temperature of incubation is as important as are the nutritional factors in macroconidial development of *M. au-*

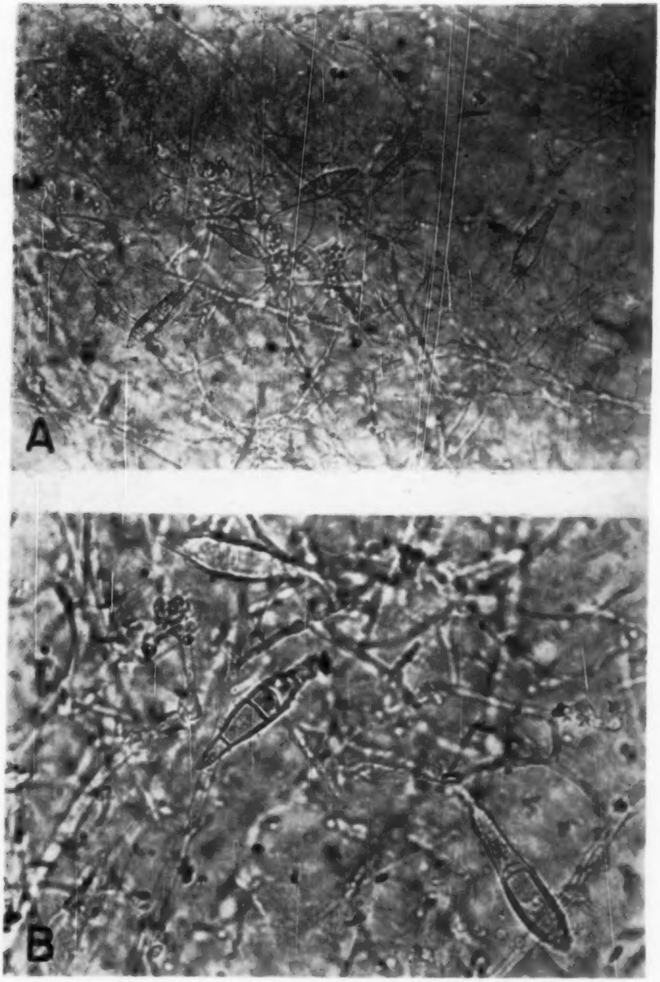

FIG. 1. *Microsporum audouini*. A. Macroconidia of isolate No. *44* on basal medium plus yeast extract. Slide culture preparation incubated at 25–27° C. Magnification × 210. B. Same. Magnification × 400.

douini. The suggestion seems valid that some of the difficulty encountered in diagnostic laboratories in the demonstration of macrospores in fresh strains of *M. audouini* is due to the common practice of incubation at unregulated temperature. So-called "room temperature" is subject

to wide variations, depending on such diverse conditions as climate, seasons of the year, heating systems, location of the laboratory rooms, etc., and therefore does not define the actual temperature of incubation. The term "room temperature" should be omitted from scientific reports. The fact, however, that *M. audouini* is deficient in certain factors essential to profuse growth and to the development of macroconidia except under optimum conditions should also not be overlooked by the diagnostic laboratory.

SUMMARY AND CONCLUSIONS

Five of 10 isolates of *M. audouini* produced a few macroconidia on honey agar with incubation at 25 to 27° C and none formed these spores at a temperature which fluctuated for the most part between 29 and 32° C with the extreme temperature being 27 and 34.5° C. Addition of yeast extract to the medium promoted a vigorous development of macrospores at 25 to 27° C, nine of the isolates forming macroconidia, while at 29 to 32° C the yeast extract had little influence, only one of the 10 isolates forming these spores.

Nine of 10 isolates produced macrospores on the basal medium plus yeast extract with incubation at 25 to 27° C, whereas at 29 to 32° C only two isolates developed more than an occasional macrospore.

The crystalline vitamins and/or amino acids present in yeast extract (Difco) in the amounts or combination used had little, if any, effect upon macroconidial formation of *M. audouini* regardless of temperatures of incubation.

A temperature range of 25 to 27° C is conducive to macroconidial production of *M. audouini* on honey agar or basal medium plus yeast extract; a range of 29 to 32° C is definitely inhibitory. The possible effect of fluctuating temperatures, however, has not been overlooked. Observations under controlled conditions of temperature are continuing.

Factors or a combination of factors essential to the development of macrospores of *M. audouini* are present in the desiccated yeast extract (Difco). Whether or not the crystalline organic components of yeast extract are lacking in some substance or substances or were not used in the proper amounts or combinations necessary for stimulation of macroconidial formation needs further investigation.

DIVISION OF LABORATORIES AND RESEARCH,
NEW YORK STATE DEPARTMENT OF HEALTH,
ALBANY 1, NEW YORK

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VARIATION IN CULTURAL CONDITIONS AND ITS EFFECT ON HYPHAL FUSION IN CORTICIUM VELLEREUM[1]

R. J. Bourchier

(with 2 figures)

The identification of cultures made from decayed wood is an important problem in forest pathological research. While the techniques designed by Nobles (**10**) for the identification of cultures are accurate in the hands of an experienced worker, these procedures are time consuming and a quick method of identification is, therefore, desirable. The hyphal fusion criterion (**3, 12**) has recently received attention from forest pathologists and others (**1, 4, 7, 11**) as a possible rapid method of identifying cultures of wood-rot fungi.

The principal difficulty in adapting this technique to the identification of many cultures is the failure of certain fungi to form fusions under the experimental conditions employed. This fact has made the interpretation of negative results risky and has restricted the usefulness of the test (**4, 11**). Cabral (**4**) has suggested varying the cultural conditions in an effort to stimulate fusion in refractory species. If conditions could be developed for each species in a collection of named cultures under which intraspecific fusion would consistently occur, then negative results using an unknown with a named culture could be interpreted with confidence and the conclusion drawn that the two cultures belong to different species.

This study was designed to examine the hyphal fuson behaviour of one test fungus, *Corticium vellereum* Ellis & Cragin, when grown under various cultural conditions.

EFFECTS OF pH, TEMPERATURE, AND MEDIUM FORMULA ON HYPHAL FUSION

Methods: Two isolates of *C. vellereum* from the stock culture collection of the Calgary Forest Biology Laboratory were selected as test

[1] Joint contribution from the Forest Biology Division, Science Service, Department of Agriculture, Ottawa, Canada (Contribution No. 313), and the Department of Botany, University of Alberta, Edmonton, Alberta. Part of a thesis presented in October, 1955, to the University of Alberta in partial fulfillment of the requirements for the Degree of Master of Science.

organisms. This fungus grows well in culture and preliminary work indicated that hyphal fusions in this species were easy to observe.

The Calgary stock culture collection is carried on malt agar prepared according to the following formula:

Difco Bacto powdered malt extract broth	20 gm
Difco Bacto agar	20 gm
Distilled water	1000 cc

Prior to fusion tests, the isolates were transfered from stock and grown for one week at 20° C on petri plates containing 20 cc of the above malt agar.

Three culture media used to test the effect of different media on the formation of hyphal fusions were prepared according to the following formulae:

Malt Agar (as above)	
Potato Dextrose Agar	
Difco Bacto potato dextrose agar	39 gm
Distilled water	1000 cc
Carrot Dextrose Agar	
Difco Bacto dextrose	20 gm
Difco Bacto agar	20 gm
Carrot extract from steaming 200 gm carrots in 500 cc distilled water for ½ hour	500 cc
Distilled water	500 cc

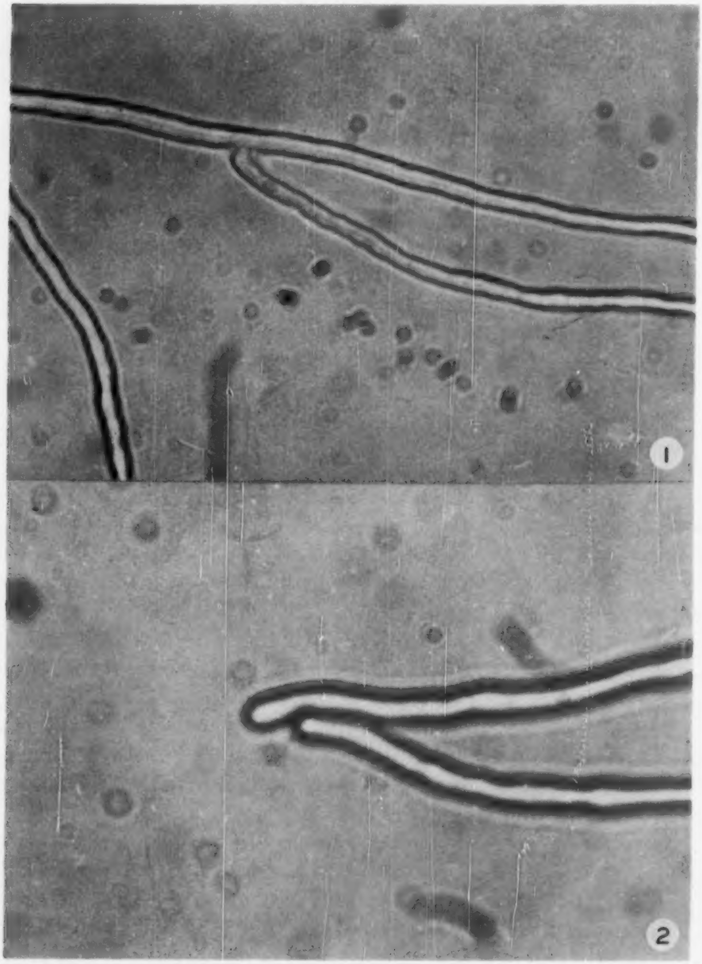
In testing the effects of the pH of the culture media on the formation of hyphal fusions, equal aliquots of each of the three media were adjusted to the pH values of 5.0, 6.5, and 8.0, ± 0.1 pH units, using N/10 HCl or N/10 NaOH as required. To these adjusted portions of media, buffer was added, made up of varying proportions of M/3 H_3PO_4, M/3 KH_2PO_4, and M/3 K_2HPO_4 solutions. Twenty percent of the final medium in each case was buffer. The pH adjustments were carried out aseptically after autoclaving.

The pH reactions of the media were determined with a Beckman pH meter. The 3 cc test portions were allowed to cool to 40° C before pH readings were taken. Temperatures were checked with a thermocouple and Rubicon pyrometer.

Constant temperature cabinets, designed to maintain a specific temperature within 0.5° C, were set at 20°, 25°, and 30° C. As a check, a recording thermometer was placed in each cabinet.

The isolates, C2 and C3, were grown on malt agar plates for one week at 20° C to provide a sufficient supply of inoculum and to give the isolates a similar recent history. Inoculum from the week-old malt

cultures was then placed on the plates of various media and pH, and incubated at 20° C for one week. These plates were the source of inoculum for the Van Tieghem cells in which fusions were observed. Three cells at each pH and of each medium were inoculated with the C2 strain and three with the C3 strain. This gave one cell of each



FIGS. 1, 2. *Corticium vellereum*. 1. A typical hyphal fusion. 2. A hyphal contact.

medium at each pH, incubated at each of the three temperatures, 20°, 25°, and 30° C, for both the C2 and C3 strains of the test fungus. The experiment was replicated three times.

A modification of the Robak (**11**) method for observing hyphal fusions was used. Other techniques were rejected because they were either troublesome to set up (**9**) or made observation of hyphal fusions more difficult in *C. vellereum* (**5**, **7**). Pieces of inoculum of uniform size were cut from the advancing zone of one-week-old petri plate cultures using a loop of flattened chromel wire. Two pieces of inoculum were cut out at a time, the second lifting the first out of the wire loop. It was then a simple matter to slide the first off the second and to place it on the lower surface of a cover slip with the surface mycelium pressed against the glass; this cover slip was placed over a Van Tieghem cell. Humidity was maintained in the cells by placing a small amount of sterile distilled water in the bottom of the glass ring.

TABLE I

RELATIVE NUMBERS OF HYPHAL FUSIONS FORMED BY THE C3 ISOLATE OF CORTICIUM VELLEREUM UNDER VARYING CONDITIONS OF MEDIUM, PH, AND TEMPERATURE*

Medium	Number of fusions	pH	Number of fusions	Temperature	Number of fusions
P. D. A.	64.117	5.0	52.274	15	48.845
Malt	46.941	6.5	49.270	20	48.920
C. D. A.	44.087	8.0	53.598	25	57.377

* Figures are totals (transformed) and contain eight estimated values.

As hyphae grew out radially from the inoculum, they frequently formed fusions with one another. On the fourth day after the inocula were put into the cells, a sample of 20 hyphal contacts was selected by randomly moving the oil immersion objective over the growth region. These were tallied as fusion formed (FIG. 1) or contact only (FIG. 2).

The variability in different numbers of samples of 20 hyphal contacts was determined. Ten cells were inoculated from malt agar cultures of *C. vellereum* and incubated at 20° C for one week. Three samples of 20 hyphal contacts from each cell were tallied for the presence or absence of fusions. A total of 30 samples of size 20 were examined; this approximates the 27 cells examined for each value of each factor in the experiment. The mean number of fusions was 1.10 with a standard error of 0.03; well within acceptable limits.

RESULTS: An examination of the basic data revealed the loss of eight cells of the C3 isolate and 21 of the C2 isolate due to contamina-

tion or desiccation. Because of these excessive losses, it was not possible to analyse the data for the C2 isolate.

The data for the C3 isolate are presented in TABLE I. The technique for estimating missing data outlined by Goulden (**5**) was employed for the eight missing cells. An analysis of variance, using the square root transformation, revealed no significant interactions between the three factors. Differences due to medium formula were significant at the 1% level. Temperature variation produced differences in hyphal fusion behaviour significant at the 5% level, while differences in fusion behaviour attributable to pH variation were not significant.

EFFECTS OF CULTURE MEDIUM CONCENTRATION AND pH

Previous work has shown that culture media low in nutrients stimulate hyphal fusion in certain fungi (**8**). To determine whether this conclusion was applicable to *C. vellereum,* the hyphal fusion behaviour of this species on three concentrations of potato dextrose agar at three pH values was examined.

METHODS: Bacto Difco potato dextrose agar was used in three concentrations; 24 gm, 48 gm, and 72 gm in 1200 cc of water. Each concentration was divided into three portions, the pH of each was adjusted to 5.0, 7.0, and 8.3, and the appropriate buffer added before autoclaving. After autoclaving, the pH values were 5.1, 6.7, and 7.1 respectively.

Isolates of the C2 and C3 strains of *C. vellereum* were grown in petri plate culture on the above media for three weeks prior to setting up of the cells for hyphal fusion examination. The fungi were transferred to fresh media weekly during this growth period.

Three cells for each combination of conditions were set up at the end of the third week of growth. These cells were examined when four days old and a sample of 20 contacts and fusions made for each, as outlined previously.

RESULTS: The data on the relative numbers of fusions formed under the various conditions are summarized in TABLE II.

There were two missing cells in the C3 series and one in the C2 series. These missing data were estimated with the Goulden technique (**5**) and the square root transformation was once again employed before the material was subjected to the analysis of variance. The latter analysis revealed no significant interaction between the two factors for either of the isolates, although there was a strong suggestion of significance in the case of the C3 isolates. Changes in medium concentration produced statistically significant (5% level) effects on the hyphal fusion behaviour of the C3 isolate but not on the C2 isolate. No significant differences

TABLE II

RELATIVE NUMBERS OF HYPHAL FUSIONS FORMED BY CORTICIUM VELLEREUM UNDER VARYING CONDITIONS OF MEDIUM CONCENTRATION AND pH *

Conc.	C3 Isolate				C2 Isolate			
	pH			Total	pH			Total
	5.1	6.7	7.1		5.1	6.7	7.1	
2%	1.577	4.471	5.725	11.773	4.245	3.326	5.109	12.680
4%	5.102	5.102	3.061	13.265	2.765	2.397	4.796	9.958
6%	7.295	6.728	5.328	19.351	2.397	4.796	2.765	9.958
Total	13.974	16.301	14.114		9.407	10.519	12.670	

* Figures are totals (transformed).

due to pH variation were discovered, corroborating the previous experiment.

The disparity between the two isolates warranted further analysis and the data were compared using "Student's T" test. A difference in fusion behaviour between the C2 and C3 strains, significant at the 1% level, was revealed.

GROWTH AND FUSION REQUIREMENTS COMPARED

Medium formula produced a detectable effect on hyphal fusion behaviour in the C3 strain of *C. vellereum;* the highest number of fusions occurred on potato dextrose agar. It was considered of interest, therefore, to compare the growth rates of the test organism on the three media employed in the fusion experiment.

METHODS: The three media were prepared and adjusted to a uniform pH of 4.5. Thirty petri plates of each medium were inoculated

TABLE III

AVERAGE DIAMETER IN CM OF 30 PETRI PLATES EACH OF THE C2 AND C3 STRAINS OF CORTICIUM VELLEREUM GROWING ON VARIOUS CULTURE MEDIA

Medium	C2			C3		
	Days after inoculation			Days after inoculation		
	4	7	12	4	7	12
P. D. A.	1.90	3.54	7.06	2.32	4.28	7.66
C. D. A.	2.46	4.45	8.15	2.57	4.66	8.03
Malt	1.66	2.80	5.30	2.01	3.83	7.11

with each strain and incubated at 20° C. The inoculum was taken from malt plates grown for six days at 20° C. On the fourth, seventh, and twelfth days after inoculation, the growth on the plates of various media was measured along two radii at right angles.

RESULTS: The analysis of variance showed significant differences (1% level) in the growth rates on the three media (TABLE III).

It is evident that the culture medium producing the greatest number of fusions in the C3 strain (potato dextrose agar) is not the most suitable for growth.

DISCUSSION AND CONCLUSIONS

In an earlier unpublished report (**1**), it was stated that temperature variation did not have a significant effect on fusion behaviour. However, this conclusion was based on the analysis of untransformed data and the subsequent use of the square root transformation increased the sensitivity of the statistical test to reveal the importance of temperature in the hyphal fusion behaviour of *C. vellereum.*

Culture medium formula has an important effect on hyphal fusion. These findings are compatible with those of Cabral (**3**), who was able to observe hyphal fusions between dikaryotic isolates of *Merulius lacrymans* Jacq. ex Fries only when they had grown on carrot agar prior to testing.

While the effect of pH variation on hyphal fusion behaviour was not significant in these experiments, it appears to have some effect on this phenomenon. A strong trend was noted suggesting the importance of the interaction of pH and culture medium concentration.

These data show that different isolates of the same species are not the same in their hyphal fusion behaviour when grown on potato dextrose agar. In previous work (**1**), an analysis of untransformed data failed to reveal this important fact. While it was impossible to compare the two isolates when grown on other media because of missing data for the C2 strain, it is reasonable to expect this difference in fusion behaviour to show up on other culture media as well. These results mitigate against the possibility of developing optimum fusion conditions for a species as a whole. At best, such an optimum would be a compromise for the various strains encountered in its development.

Laibach's conclusion that culture media low in nutrients stimulate hyphal fusion held true for the C2 strain of *C. vellereum* but not for the C3 strain when they were grown on three concentrations of potato dextrose agar. A concentration of this medium, approximately one and one-half times that commonly used in fungus culture work, and the strongest tested, produced the greatest number of fusions in the C3

strain. These results emphasize further the variability in fusion behaviour encountered between the two strains of the one species, *C. vellereum*.

The possibility of using the hyphal fusion technique for mass culture identification in wood decay projects depends on the ability to produce fusions consistently between different isolates of the same species. One approach to this goal, that of developing optimum culture conditions for hyphal fusion for the species commonly encountered, is apparently closed by the variability in fusion behaviour of different strains of the same species. The hyphal fusion technique is useful in certain cases but the writer agrees with Robak (**10**) in concluding that its value in a large scale culture identification program is of a supplementary nature, useful only in those cases where fusion occurs.

SUMMARY

Because of the lengthy procedures and experience required to identify cultures of wood-rot fungi, a project was begun in 1954 to gain more information on the value of the hyphal fusion technique for culture identification.

Culture medium formula and the temperature at which the fungi were grown each had statistically significant effects on the fusion behaviour of one strain of *Corticium vellereum*. Potato dextrose agar medium consistently produced the greatest number of fusions in this fungus, while 25° C was the temperature most conducive to fusion.

The concentration of potato dextrose agar medium had a significant effect on fusion formation.

No significant differences due to pH variation were revealed, although a trend suggesting a significant interaction between potato dextrose agar concentration and pH was noted.

A significant difference in hyphal fusion behaviour was detected between two strains of *C. vellereum*.

It is concluded that, because of the variability found between two strains and the resulting difficulty in developing optimum conditions for hyphal fusion in a species, the hyphal fusion technique is not amenable to development for use in identifying large numbers of cultures. Rather, its use is of a limited and supplementary value only.

ACKNOWLEDGMENTS

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FOREST BIOLOGY LABORATORY,
102 ELEVENTH AVENUE, EAST,
CALGARY, ALBERTA, CANADA

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PHYSIOLOGICAL AND GENETIC ADAPTABILITY IN THE FUNGI [1]

ALFRED S. SUSSMAN [2]

The past 50 years have witnessed the explosive development of genetics and physiology both as separate disciplines and as applied to mycology. In physiological terms we have seen the transition from the lag phase to the logarithmic phase of the development of these disciplines. Paradoxically, perhaps, the more intensively these have been studied the more their boundaries have blurred and merged and the more their data have coalesced with those of their sister fields of morphology, taxonomy and cytology. It is this welding of the descriptive and analytical sciences that I consider to be one of the most important contributions of the work of the past 50 years and which will serve as the springboard for my presentation today.

ADAPTATION DEFINED

The mycologist of today is confronted, as were his predecessors, with an heterogeneous array of organisms characterized by extreme variability both as to genotype and phenotype. There are exceptions, of course, but one immediately suspects homogeneity within a fungous taxon to be symptomatic of insufficient sampling. Such heterogeneity has insured the success of this group by virtue of the adaptiveness bestowed upon it. It should be noted that I consider adaptation to describe "the totality of the various processes of change which confer on an organism fitness to its environment" (Stanier, **1953**). Whether such fitness is measured by ability to leave offspring, to diffuse through many habitats, or to overtax the taxonomist, it seems clear that the fungi pass the test.

At this point qualifications must be introduced into the use of this much-abused term, for it can best be described as being the resultant

[1] Paper presented as part of a symposium, "Some Contributions of Research in Microbiology to Fundamental Biological Problems," Mycological Society of America and Microbiological Section of the Botanical Society of America, Storrs, Connecticut, August 29, 1956.

[2] Paper No. 1065 from the Department of Botany, University of Michigan, Ann Arbor, Michigan.

of both genotypic and phenotypic components. Its genotypic aspect is represented by the change induced over relatively long periods of time by gradual environmental shifts. On the other hand, the phenotypic component is that manifested when environmental alterations induce changes with the span of one or a few generations.

Therefore, accepting the fact of the potent versatility of the fungi, I would like to proceed to the question of why this should be and its concomitant, namely, how have physiology and genetics aided in providing an answer?

THE CELL SURFACE

It is fitting, perhaps, to begin the discussion of adaptiveness with the cell surface, which is the gateway of the environment to the cell and the reciprocal as well. Characteristic of microorganisms is a resistant or dormant stage which permits them to remain viable under unfavorable environmental stress. While some fungi are endowed with a special coat during a spore stage, many can survive in the vegetative condition. The cell surface helps to tide the fungus over dangerous periods which may be very long when compared with their generation time. Moreover, it is becoming apparent that this structure is an active rather than an inert component of the cell. Therefore, analysis of its morphology and chemical nature becomes pressing and fortunately some remarkable technical advances have assisted in this task.

These advances have permitted the visualizing of details of the cell surface which should prove of fundamental importance to the descriptive mycologist as well as to the physiologist. The work of Hess and Schantz (**1956**) is an excellent example of the results obtainable with newer techniques in electron microscopy wherein the surface of rust spores was studied by a gum-replica technique. That the chemistry of the cell surface is a virtually untapped reservoir of taxonomic characters is being increasingly recognized through the work of Frey (**1950**), Kreger (**1954**), Blank (**1954**) and others. The presence of cellulose instead of chitin is a meaningful distinction in the Phycomycetes but it is less well known that other polymeric polysaccharides have been demonstrated to occur and may prove to be of taxonomic as well as of medical significance. In the former category is the work of Prentice and Cuendet (**1954**) showing the presence of d-arabitol, a sugar of rare occurrence in nature, in uredospores of *Puccinia graminis tritici*. The chemical analysis of the capsular materials of *Cryptococcus neoformans* (Evans and Theriault, **1953**) and *Blastomyces dermatiditis* (Blank, **1954**) and the direct visualization of the capsule of *Histoplasma capsu-*

latum (Ribi and Salvin, **1956**) have established the value of these techniques in medical mycology.

At this point some mention of work on the cell wall of other groups of microorganisms is pertinent. This concerns the discovery by Work (**1950**) of a new amino acid, diaminopimelic acid, in bacterial hydrolysates and the subsequent finding by Salton (**1953**) that this substance is one of the constituents of the cell wall. It was found only in those bacteria that were related by physiological and nutritional similarities and in the blue-green algae, a possibly crucial bit of evidence linking this group to the bacteria. Another approach was taken by Nicolai and Preston (**1952**) in their studies of the Chlorophyceae, wherein the nature of the cell wall of various of these algae was studied.

Although such information is still scarce for the fungi some work has been reported. There is the detailed report of Frey (**1950**), alluded to earlier, and those of Kreger (**1954**) and Kreger and Meeuse (**1952**). With respect to the x-ray powder diagram method of determining the structure of cell walls, the work reported in the last-named paper has removed some of the difficulties associated with the method.

Although the cell surface may perform functions like adsorption, which are independent of whether the cell is alive or killed, there is an increasing weight of evidence to suggest more active roles. These are made manifest by the presence of enzymes which appear to be closely bound to the cell surface. In view of the pronounced ability of fungi to degrade insoluble substrates and to liberate extracellular enzymes of other kinds (Fåhraeus, **1952**) it is perhaps not surprising that this is so. In this connection the liberation of a high molecular weight glucosamine derivative into the medium by fungi like *Neurospora crassa* and various of the Aspergilli (Blumenthal *et al.*, **1955**) would seem to be extremely unlikely except by means of an enzyme on the surface. A final *a priori* reason for expecting surface localization would be the probability that such enzymes might be necessary as the means by which the surface itself is maintained and extended.

An extensive monograph on this subject has been written by Rothstein (**1954**) so that it would be superfluous to repeat many historical details. However, it might be worth discussing the criteria used in assigning a superficial locus to an enzyme. Some of the first work in the field (Willstäter and Lowry, **1925**) disclosed that yeast cells treated for about an hour with 0.2 to 0.3 N H_2SO_4 lost their invertase and maltase activity; at the same time they retained their ability to grow and ferment glucose. A similar argument was advanced by Wilkes and Palmer (**1932**), who found that the invertase activity of living yeast is

markedly susceptible to small changes in environmental pH. Myrbäck and co-workers (**1936, 1937, 1943**) described similar effects in the case of the trehalase and lactase of yeast. It could be argued that pH was affecting permeability to substrate rather than enzyme activity, but the work of Demis, Rothstein and Meier (**1954**) seems to rule out this possibility. That enzymes other that carbohydrases are to be found on the cell surface was shown in the case of ascorbic acid oxidase of *Myrothecium verrucaria* (Mandels, **1953b**), a phosphatase of yeast (Rothstein and Meier, **1948, 1949**) and a diphosphopyridine nucleotidase of conidia of *Neurospora crassa* (Zalokar and Cochrane, **1956**).

The recent development of enzymatic techniques for the selective removal of bacterial cell walls by lysozyme (Weibull, **1953**) opens the way for refinements in the study of this problem. This enzyme is so specific that, in a medium of the right tonicity, the naked protoplast, although subject to every vicissitude of the environment, retains most of its synthetic and metabolic capacities. It is possible that the specific enzymatic removal of the cell wall, if accompanied by the removal of certain enzymes, would be strong *prima facie* evidence for surface localization. In this connection, the observations of Lowry, Sussman and Heidenhain (**1956**) on the susceptibility of ascospores of *Neurospora tetrasperma* to lysozyme offer promise for such an approach in the fungi.

This rapid survey of the periphery of the cell has disclosed that adaptive advantages accrue from the resistant nature of the wall, a feature conferring survival value during hard times. In addition, it is possible that the wide range of substrates attackable by this group is attributable, at least in part, to the surface localization of carbohydrases and other enzymes. Finally, although the function of these materials is still unknown, some selective advantage might be obtained from the excretion of substances like the glucosamine-polymers mentioned before. If a guess is to be permitted, perhaps the fact that many synthetic basic polymers are toxic (Katchalski *et al.*, **1954**; Rubini *et al.*, **1951**) may argue for an antibiotic role during the cyclic microsuccessions of a fungal environment.

THE CYTOPLASM

Induced enzymes. The classic paper of Perquin (**1932**), which pointed the way to modern metabolic studies via the shake culture method, also provided a clear example of the adaptive response of a fungus to its environment. It was shown that the nature of the inorganic nitrogen source furnished *Aspergillus niger* determined whether

a sugar acid or CO_2 was recovered among the products. The next step in the study of such metabolic adaptation was to pinpoint it in terms of the enzymatic reactions involved. A short time before this work had been reported, Karstrom (**1930**) had taken another approach wherein a strain of *Betacoccus arabinosaceus* was grown in media containing different sugars. He then tested these cells for their ability to ferment arabinose, galactose, maltose, and lactose and found that they could do so only if they had been grown in a medium containing these sugars. Since he had used cells that were deprived of a nitrogen source,

TABLE I

"INDUCED" ENZYMES REPORTED FROM THE FUNGI

Enzyme	Organism	Inducer	Reference
Laccase	Polyporus sp.	tyrosine, p-hydroxy benzoic acid	Fåhraeus and Lindeberg (**1953**)
Protocatechuic acid oxidase	*Neurospora crassa*	m-hydroxy benzoic acid, protocatechuic acid, gallic acid	Gross and Tatum (**1955**)
Tyrosinase	*Glomerella cingulata*	unknown	Sussman and Markert (**1953**)
Cellulase	Tricholoma	cellulose	Norkrans (**1950**
Amylase	*Aspergillus flavus, A. terreus*	starch	Goodman (**1950**)
Pectase	*Botrytis cinerea, Aspergillus niger*	pectin	Gäumann and Böhni (1947 a, b)
Raffinase	*Saccharomyces fragilis*	raffinose	Davies (**1953**)
β-galactosidase	*Ophiostoma multi-annulatum*	lactose	v. Hofsten (**1956**)
Invertase	*Saccharomyces Rouxii*	sucrose	Terui and Sase (**1955**)
Galactokinase	*S. cerevisiae*	galactose	Trucco, Leloir and Mittleman (**1948**)
Galactowaldenase	*S. cerevisiae*	galactose	Caputto, Leloir, Trucco, Cardini and Paladini (**1949**)

as well as short-time experiments, the possibility of mutation and subsequent selection was obviated. This, then, was a phenotypic phenomenon which he attributed to the formation of so-called "adaptive enzymes." (Because of the looseness involved in the use of the term adaptive, the term of choice would seem to be "induced enzyme biosynthesis.") In contrast to those enzymes whose titer varied in response to the composition of the medium, were the "constitutive enzymes" like those responsible for the fermentation of glucose, fructose and mannitol. TABLE I provides several observations of the same kind

that have been made among the fungi, so that it is clear that this is not an isolated phenomenon, nor is it restricted to one type of enzyme activity.

Moreover, as the work of Slonimski (**1953a**) has shown, whole enzyme systems may fluctuate in response to environmental factors. He demonstrated that the usual complement of cytochromes c, b and a was replaced by cytochromes a_1 and b_1 after exposure to anaerobiosis. Lindenmayer (**1955**) has been able to show that even these residual respiratory pigments virtually disappear under conditions where yeast grows anaerobically. That these are not the only enzymes affected was shown by the fact that the titer of many respiratory enzymes decreased while that of the fermentative ones rose. This transformation was largely reversible upon exposure of the cells to air. Slonimski also noted that the respiratory enzymes were associated with particles that sedimented at 30,000 × g, except for malic dehydrogenase.

Other environmental influences upon the development of enzymatic activity include the effect of temperature upon pectase activity in *Botrytis cinerea* (Gäumann and Nef, **1947**), tyrosinase activity in *Neurospora crassa* (Horowitz and Fling, **1953**) and in *Glomerella cingulata* (Sussman, Caughey and Strain, **1955**), and cellulase in *Neurospora* (Hirsch, **1954**). Light, too, has been shown by Schaeffer (**1953**) to decrease melanogenesis and tyrosinase activity in the mycelium of a mutant of *Neurospora crassa*.

Interesting ecological implications derive from the enzymatic responsiveness of the fungi to their environment. For example, Hirsch (**1954**) suggests that the elaboration of a cellulase by *Neurospora* at high temperatures, but not at low, is a contributing factor to this group's survival in the wild state mainly in the warmer climates. Another instance is that discussed by Sussman, Caughey and Strain in which certain strains of *Glomerella cingulata* produce melanoid pigments at 20° C but not at 30°. In this case, distinct survival value attaches to such a mechanism whereby the amount of heat absorbed by the organism, which is a direct function of the intensity of its pigmentation, is regulable by the temperature of its environment. This may, in fact, serve as a model to explain the distribution patterns of animal species that are white at their origin but which become progressively darker as they spread into more temperate climates.

To be sure, there are several possibilities involved in the interpretation of data suggesting induced enzyme synthesis. These include changes in permeability, mutation, and the presence of inhibitors as well as the original suggestion of the synthesis of new enzymes. Several

reviews of the literature in this field are available (Monod, **1952**; Spiegelman, **1951**, etc.) as well as a book which records the proceedings of a symposium on this subject (Gale and Davies, **1953**).

However, there are an increasing number of cases wherein the concept of induced enzymes developed previously needs revision. The data of Barrett, Larson and Kallio (**1953**) illustrate this point. If one exposes fumarate-grown cells of *Pseudomonas fluorescens* to fumarate the substrate is immediately oxidized. However, such cells display the typical inductive lag when citrate is added as substrate. On the face of it, this is a typical case of induced enzyme biosynthesis but this conclusion turned out to be unwarranted when extracts of these cells were prepared and tested for their ability to oxidise citrate. When this was done, there was no significant difference in the rates at which citrate disappeared in extracts prepared from fumarate- or citrate-grown cells. These conclusions have been confirmed by Kogut and Podovski (**1953**) and extended to *Azotobacter agile* by Repaske and Wilson (**1953**). The explanation of these effects may well lie in the suggestion by Monod (**1956**) that distinct and specific uptake systems may exist for each type of carbohydrate metabolized by an organism. Therefore, such *y* systems could be induced concomitantly with a *z* system which, in the above case, would correspond to the enzymes responsible for the oxidation of citrate. If Monad is correct, the data of Larson *et al.* would be explicable in terms of the necessity for the induction of a "citrate-transport" system which would be formed independently of the oxidative system and which would serve to bring the substrate into the cell. Therefore, the properties of "cryptic" mutants, wherein divergences between the enzyme activity of whole cells and extracts have been found, could be explained in like terms. In line with the previous discussion of surface-localized enzymes it seems that the *y* system(s) might logically be sought at an interface located within or on the cell surface.

The genetic nature of cytoplasmic particulates. Another property of the *y* system, namely, its ability to perpetuate itself in a growing population, introduces the next subject: are there indeed self-perpetuating elements in the cytoplasm and, if so, what is their function?

The delineation of such particulates in the cytoplasm of higher organisms has proceeded along two main lines: first, through their visual identification by electron microscopy and classical cytological techniques, and second, by the parallel examination of their biochemical properties. The evidence for the identity of the visible structures and those showing particular types of metabolic activity is very compelling and has been

a triumph of the combined approach of the different disciplines (Porter, **1955**). In addition, the ground substance, or reticulum, from which microsomal elements may be derived, has also been the subject of rewarding investigation (Palade, **1955**). It has, therefore, been tempting to extrapolate from this detail obtained with materials of higher plant and animal origin to the microbial cell. However, the dangers involved in such an extension to the bacteria, of data derived from other organisms (Stanier, **1954**), serve as a warning to the unwary.

What details are known were derived by a combination of statistical techniques like the Poisson series with biochemical of physiological data. On these bases an imposing list of such entities has been assembled from a variety of organisms. These include Kappa, Sigma and Gamma, that fascinating fraternity of factors from *Paramecium* (Preer, **1948**), *Drosophila* (Ephrussi, **1953**), and *Blastocladiella* (Cantino and Horenstein, **1956**). In addition, there are the petite particles of yeast (Ephrussi, **1952**), the *y* factor of bacteria (Monod, **1956**), the plastogenes of higher plants (Rhoades, **1946**), and others. For the purposes of this discussion I should like to borrow the terminology of Lederberg (**1952**), who, with great economy, uses the term "plasmid" to describe "any extrachromosomal hereditary determinant."

Some of the strongest arguments for the reality of such determinants have come from the work with yeast performed by Ephrussi (**1952**). This worker discovered that when baker's yeast was plated out after treatment with acriflavin,[3] almost all of the resultant colonies developed at a very slow rate and were of much smaller size than the parental strain. Accompanying this difference in morphology was a large number of other changes, some of which are listed below:

1. a total absence of a cyanide-sensitive respiratory system, and of aerobic fermentation.
2. abolition of the Pasteur mechanism.
3. total absence of cytochromes a and b, cytochrome oxidase, succinic dehydrogenase, DPNH cytochrome c-reductase, and reduced amounts of malic dehydrogenase.
4. increased amounts of alcohol dehydrogenase and cytochrome c.

More recently it has been shown by Slonimsky (**1953b**) that euflavin, the most effective of the acridine derivatives used, blocks the synthesis of cytochrome oxidase rather than its activity once formed. Borrowing from the work mentioned earlier on the effect of aeration upon the

[3] Acriflavine is a mixture of 2,8-diamino-n-methyl acridinium chloride (euflavine) and 2,8-diaminoacridinium chloride (proflavine).

enzyme complement of yeast cells, we are drawn to the conclusion that the acridine compounds inhibit the synthesis of a plasmid which is the site of enzymes associated with terminal oxidation. Support for this conclusion has also been derived from crossing experiments performed by this same group of workers.

Although the evidence for this yeast plasmid is growing, direct proof in terms of its detection microscopically is still lacking. Nevertheless there is hope that with the extension of some recently devised techniques even this can be accomplished. For example, Chao and Schachman (**1956**) have identified a particle containing RNA which sediments at a rate of 80 Svedbergs (1 S is 10^{-13} cm/sec/unit field). These particles comprise roughly one-quarter of the weight of the original yeast and are relatively rich in RNA but lack DNA. These workers have calculated their diameter to be between 21–24 mμ in size, if they are assumed to be spherical, and electron micrographs of these elements confirm this estimate. Although it is still too early to relate these particles to any previously reported plasmids a promising approach has been developed in their analysis.

Other yeast particulates having biochemical capacity have been isolated. These include a fraction that centrifuges down between 25,000–60,000 $\times$ g which appears to incorporate labeled acetate into fatty acids and lipids (Klein and Booher, **1956**) and is of the size of the 80-S particle. Moreover, Singer and coworkers (**1956**) have purified a succinic dehydrogenase from particles sedimenting in the same range of speeds. Whether these entities are identical, or whether several organelles of similar structure but different function exist will be an interesting question for the future.

Cytoplasmic systems resembling that of yeast have been reported in other fungi, like the *poky* mutant of *Neurospora crassa*. Tissieres and Mitchell (**1954**) have shown that a deficiency in this organism's cytochrome system is inherited maternally and can best be ascribed to a plasmid. In addition, the work of Cantino and Horenstein (**1956**) on a plasmid they have named gamma may have important implications as far as the inheritance of sex in *Blastocladiella* is concerned.

Enzyme localization. It has been mentioned before that the enzyme complement of plasmids has been used in their characterization. Although such work has not been intensively pursued in the fungi, scattered references do exist. The most detailed of these concerns the work of Slonimski (**1953a**) to which allusion has been made previously. In addition, the particulate localization of cytochrome oxidase in *Myrothecium verrucaria* (Darby and Goddard, **1950**) and *Glomerella cingu-*

lata (Sussman and Markert, **1953**) plus the recent report of Bonner and Machlis (**1956**) on the oxidative enzymes of *Allomyces macrogynus* offer a parallel to the work in other organisms.

On the other hand, the work of Slonimski (**1953a**) showed that malic dehydrogenase activity is greatest in the supernatant fraction of the yeast preparation. If it can be assumed that this enzyme has not simply been "rubbed off" the plasmid by preparative techniques this would appear to be at variance with many of the results in work with higher organisms. That this may not be an isolated phenomenon, however, is suggested by the work of Hilton and Smith (**1955**) on the malic dehydrogenase of *Myrothecium verrucaria,* that of Nossal (**1953**) on the fumarase and aconitase of yeast, and that of Neilson (**1956**) on the aconitase of *Aspergillus niger.* For a review of this subject in bacteria Alexander (**1956**) can be consulted.

To summarize, the cytoplasm, then, can not be construed any longer to consist of a homogeneous colloidal "substance" which bathes the all-important nucleus with the food needed for genic and protein replication. As work with the fungi has disclosed, it is an exceedingly heterogeneous element of the cell with its own capacity for both phenotypic and genotypic adaptation. Although experimental proof is still lacking, the interesting speculations of Lederberg (**1952**) on the origin of plasmids as related to intracellular parasites offer fascinating biological possibilities.

THE NUCLEUS

It is not within my province to go into a detailed discussion of the nucleus and its genetic involvements. However, its central role in heredity and reproduction demands its inclusion. Moreover, it is in the behavior of the nucleus that the fungi differ substantially from other organisms, so that we might look to this organelle for reasons for adaptive significance.

The possibilities for variations in the nuclear content of mycelia include the monokaryon, and the several types of heterokaryons discussed by Raper and San Antonio (**1954**). In a heterokaryon, nuclei differing in two or more hereditary properties multiply within a cytoplasm which is affected by the two kinds of nuclei and which, in turn, may affect them. Pressures from the external environment selectively adjust the proportions of the two kinds of nuclei by differential division at the cellular or nuclear level. Jinks (**1952**) has shown that naturally occurring heterokaryons in Penicillia confer great plasticity upon the growing mycelium. "Balanced" heterokaryons between nuclei deficient

in the ability to synthesize metabolites have shown how the equilibrium between the two types of nuclei is adjusted as the nature of the medium changes. Heterokaryosis, therefore, is certainly of importance for a colony growing upon solid materials wherein the nutritional levels change with the growth of the organism. As Pontecorvo and Gemmell (**1944**) have shown, all situations between hyphae which are almost exclusively heterokaryotic to those that are homokaryotic can be found. Segregation of the components of the heterokaryon will occur unless a selective advantage accrues to the maintenance of the two different nuclei. Adaptiveness via nuclear interactions in the heterokaryon is carried to a fine point in the fungi.

But yet another means of insuring variation is found to be superimposed upon sexual recombination in some fungi like *Aspergillus nidulans* (Pontecorvo and Roper, **1953**) or substituted for sex, as in *A. niger* (Pontecorvo *et al.*, **1953**). This phenomenon concerns the infrequent fusions of haploid nuclei of a heterokaryon at a rate of about 1×10^{-7}. The mitotic division of this heterozygous diploid then occurs with concomitant crossing-over, leading to the segregation of two alleles of certain genes. Add to this occasional accidents wherein random distribution of chromosomes at mitosis occurs, and the opportunity for the formation of a functional haploid with a new combination of genes becomes realized. The result of this curious sequence of events is precisely the same as in sexual reproduction, so that the "Fungi Imperfecti" may be imperfect only insofar as the ritual of sex is concerned, not as far as the results.

The work of Papazian (**1954**) and Raper (**1953**) has suggested other opportunities for variation through nuclear genes. In this connection I should like to mention the fascinating possibility of the interchange of factors between the nuclei in vegetative hyphae which has been studied by Papazian (**1954**). To summarize the discussion of the nuclear contribution to adaptiveness, the heterokaryon and subsidiary mechanisms which are peculiar to the fungi, supplement and sometimes supersede the usual sexual means of variation and lead to many more such opportunities.

To conclude this entire discussion, we have seen how each of the elements of the fungal cell contributes to the adaptiveness of the whole. It is in their interactions, as analyzed by the entire armamentarium of the biologist, that the advances of the next 50 years must come.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN,
ANN ARBOR, MICHIGAN

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BIOLOGICAL AND CULTURAL STUDIES OF THREE SPECIES OF PROTOMYCES

K. Tubaki

(with 3 figures)

The genus *Protomyces,* established by Unger in 1832 and based on *Protomyces macrosporus,* is the type genus of the family Protomycetaceae. This family also includes *Taphridium* Lagerh. & Juel, *Volkartia* Maire, and *Protomycopsis* P. Magn. About nineteen species of Protomycetaceae have been described. The systematic position of the family is still doubtful. All species of *Protomyces* are parasitic, causing galls on stems, leaves or fruits of Compositae or Umbelliferae and forming in the tissues of their hosts large, round, thick-walled resting chlamydospores (called sporangia by some authors) as the result of enlargement of segments of the mycelium. These spores are subepidermal or are intercellular in the underlying tissues.

Sappin-Trouffy (**1897**), Popta (**1899**), Büren (**1915**, **1922**) and Fitzpatrick (**1930**) have studied the genus cytologically or morphologically. In Japan, K. Sawada (**1923**, **1925**, **1935**, **1943**) reported seven species in Formosa, of which three were new, and S. Akai (**1939**) studied anatomically the galls of *P. pachydermus* and *P. inouyei.* Büren reported in detail the germination of chlamydospores and the conjugation and the budding of liberated spores. Virtually nothing has been reported, as far as I am aware, concerning behavior of *Protomyces* in artificial media.

It seemed therefore worth while to study species of *Protomyces* in pure culture in the hope of obtaining information regarding their life cycles and the true position of this genus among the fungi.

MATERIALS

The following materials were obtained:

Protomyces inouyei P. Hennings, Engl. Jahrb. **32**: 34. 1902.

Hab. On *Crepis japonica.* Prov. Yamaguti (I. Hino, April, 1954). Prov. Tokyo (Suematsu, May, 1954). Prov. Tiba (Tubaki, May, 1954). Prov. Tokyo (Tubaki, June, 1954 and 1955). Prov. Kyoto (S. Akai, June, 1954).

Protomyces lactucae-debilis Sawada, Trans. Nat. Hist. Soc. Formosa **15**: 78. 1924.

Hab. On *Lactuca debilis.* Prov. Kyoto (S. Akai, May, 1955).

Protomyces pachydermus Thuemen, Hedwigia **1874**: 97. 1874.

Hab. On *Taraxacum platycarpum.* Prov. Kyoto (S. Akai, May, 1955).

The diameters of the chlamydospores of these three species are as follows:

Species	Diameter in microns
P. inouyei	37–45 × 35–40
P. lactucae-debilis	35–45 × 35–40
P. pachydermus	40–50 × 37–45

The specimens listed above were stored under three different conditions: at room temperature in the dry state, at room temperature in distilled water, and in the refrigerator below 5° C. In alternate months, chlamydospores from each plant were placed in hanging drops of sterilized distilled water at 20° C and were examined for possible germination.

GERMINATION OF CHLAMYDOSPORES

The chlamydospores of these three species germinated after about seven months. Germination ratios were high from autumn until spring of the next year. The above three conditions of storage were substantially similar as to their effect in influencing germination, but storing in the refrigerator seemed most effective.

Spores commonly germinate within 7–24 days in water or on moistened filter paper; the latter method seemed better for germination. Evidently a resting period is prerequisite to germination and moisture and oxygen seemed also to be necessary. Büren has stated that alternate moistening and drying of tissues is necessary. Heat-treatment or hydrogen peroxide-treatment of chlamydospores did not, so far as tested, improve germination.

The membrane of chlamydospores of *Protomyces* is three layered, consisting of a very thick, colorless, unsculptured epispore averaging 5.5 μ in thickness and giving a cellulose reaction with chlorzinc-iodine, a very thin mesospore and an endospore. The cell contents are differentiated into a light colored peripheral layer and a darker central portion. During germination, the protoplasm of the central portion becomes denser, and soon the whole protoplasm becomes homogeneous, remaining so until the chlamydospore-walls rupture and the spore-sacs appear.

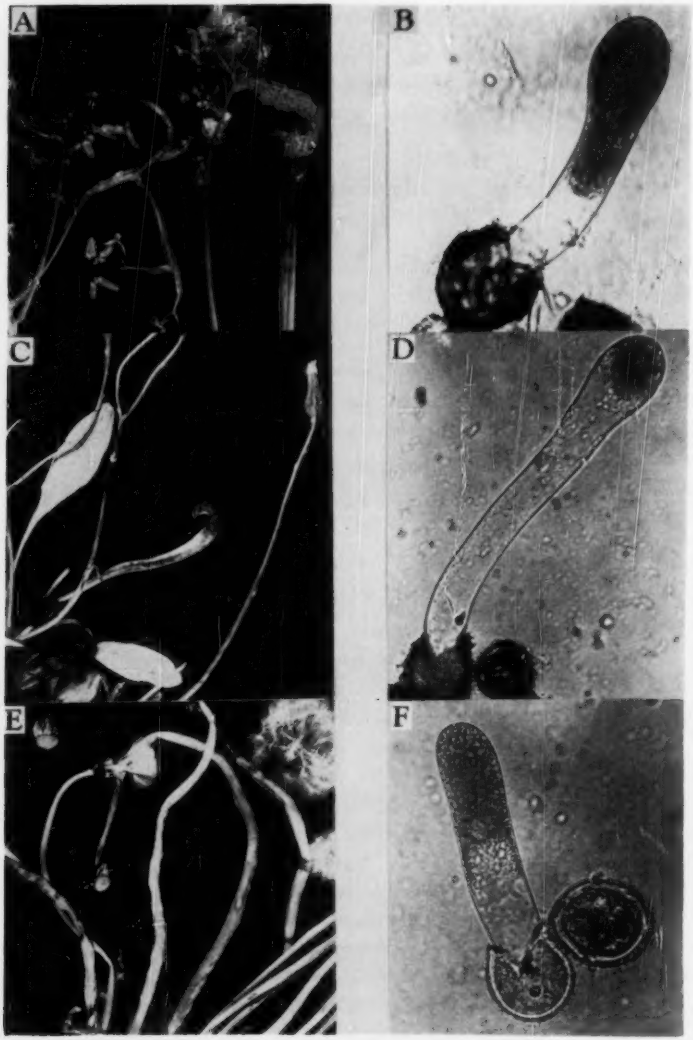

FIG. 1. *Protomyces* spp. Habit and germination of chlamydospores. A, B. *P. inouyei* on *Crepis japonica*, × 0.6; germination, × 40. C, D. *P. lactucae-debilis* on *Lactuca debilis*, × 0.6; germination, × 35. E, F. *P. pachydermis* on *Taraxacum platycarpum*, × 0.5; germination, × 30.

Gradually vacuoles appear in the denser central portion and extend into the periphery, so that the differentiation between the two portions becomes slight. When germination begins, the outer walls of the spore rupture and inner thin-walled cells (1.0–1.2 μ width) emerge as spore-sacs (asci, synasci or sporangia according to different authors). The contents of these sacs are homogeneous, turbid, and very granulated. These granules are thrust up to the upper half of the sac as the protoplasm extends and become transformed into young spores, lying irregularly in this part of the sac. These endogenous spores mass at the apex of the sac and by breaking of the latter are projected along with slimy epiplasm to distances of 200 μ or more. This discharge, due to increase of the osmotic pressure of the protoplasm, takes place even in water. These projected spore-masses are commonly 15–80(120) μ in diameter. In *P. pachydermus*—not in the other two species—the spores fuse by two's in the sacs or after expulsion and are connected by fusion tubes. This conjugation is isogamous and occurs between two adjacent cells.

After the projection of the spores onto nutrient substrata, each spore of *P. inouyei* and of *P. lactucae-debilis* buds with monopolar or multipolar budding exactly as in some yeasts; paired spores of *P. pachydermus,* on the contrary, usually begin to bud from one cell of the pair. Daughter cells may bear new buds. In all three species, very short protuberances on which new buds are produced are sometimes formed.

The length and width of spore-sacs of each species is variable, measuring as follows:

Species	Length in microns	Width in microns
P. inouyei	145–55	45–25
P. lactucae-debilis	280–100	40–20
P. pachydermus	100–45	60–30

Chlamydospores from fourteen specimens [1] collected by Sawada in Formosa could not be induced to germinate.

CULTURE METHODS

For obtaining pure cultures, the spore-fall method was the usual procedure. When chlamydospores are ready to germinate, they are

[1] *P. inouyei* Hennings on *Crepis japonica* (five specimens), Taihoku (March, 1925, 1925, 1936 and April, 1927) and Hokuto (April, 1925); *P. ixeridis-oldhami* Sawada on *Lactuca oldhami* (three specimens), Taihoku (February, 1926, 1926) and Kilung (April, 1929); *P. lactucae-debilis* Sawada on *Lactuca debilis,* Taihoku (April, 1936); *P. pachydermus* Th. on *Taraxacum formosanum,* Taihoku (May, 1935); three specimens on *Lactuca formosana,* Taihoku (May, 1926; April, 1927 and February, 1928); one specimen on *Lactuca laciniata,* Taihoku (February, 1928). All these specimens are preserved in the National Science Museum, Tokyo.

deposited on a bit of sterilized, moistened filter paper inside the upper half of a petri dish containing culture media. The dish is then incubated, not inverted, at 20° C until the colonies can be counted. In a second method the expelled mass of spores or spore-sacs containing spores is picked up from water with a pasteur pipette and transferred to the medium. Malt agar was used for isolation and for preservation of cultures. In general, cells become larger in culture than in the sacs.

GENERAL MORPHOLOGICAL CHARACTER OF CULTURES

Büren stated that the spores bud in a yeast-like manner, but do not separate from each other; however, in this investigation they were seen to bud and to separate readily. Morphologically the three species are alike except as to diameter of cells and cannot be easily differentiated. Growth on malt agar at 20° C is very slow. Colonies are dull, flat, sometimes wrinkled, pasty, restricted, with a smooth margin and cream to pale orange or pinkish in color. Cells are variable in shape, commonly ovoid, ellipsoid or cylindrical; often irregular-shaped cells containing two or more oil drops appear singly or in pairs surrounded by a capsule. In malt extract sediment is formed followed by a ring, and occasionally a few islets which easily drop to the bottom; rather firm pellicles are formed in old cultures. In slide cultures, pseudomycelium is usually not formed, but sometimes a few branched chains of cells may be seen. The range in size of cells of the three species on malt agar after 10 days at 20° C is as follows:

Species	Size of cells on malt agar, in microns
P. inouyei	$8.0–18.0 \times 4.5–9.5(11.5)$
P. lactucae-debilis	$6.5–12.5 \times 4.0–6.5$
P. pachydermus	$9.0–18.0(20.0) \times 4.5–10.5(13.0)$

Conjugation of cells has not been observed in agar culture nor in aqueous suspensions of cultivated cells of *P. inouyei* and *P. lactucae-debilis,* but in old cultures of *P. pachydermus* conjugated cells, exactly like those formed in spore-sacs, are to be seen.

PHYSIOLOGICAL CHARACTERS

Cultures of the three species were tested for utilization of various compounds of carbon and of nitrogen by the auxanographic method of Lodder and Kreger-van Rij; and also by observation of growth in liquid media. All cultures were made in duplicate and all experiments were repeated twice. Further repetitions were made where compounds were

refused. All species were oxidative and no fermentative ability was observed.

Assimilation of carbon sources: All species utilized sucrose, maltose (weakly), glucose, fructose, mannite and succinic acid. All species refused galactose, xylose, lactose, rhamnose, arabinose, inulin, starch, gly-

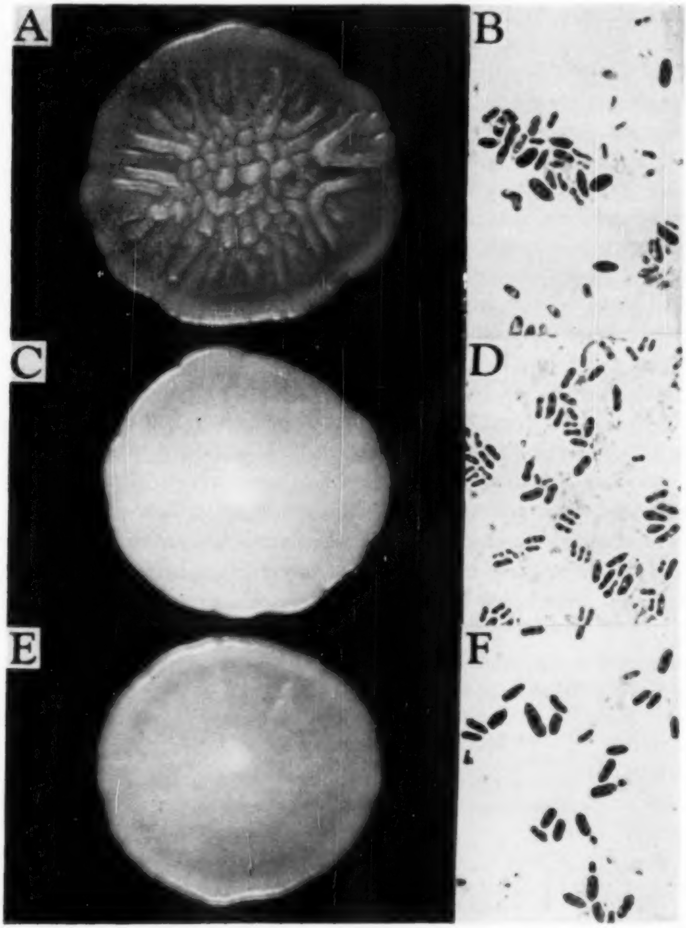

FIG. 2. *Protomyces.* Colonies on malt agar after 20 days, × ½, and budding phases stained with haematoxylin, × 250. A, B. *P. inouyei.* C, D. *P. lactucae-debilis.* E, F. *P. pachydermis.*

cogen, sorbitol, ethanol and dulcitol. *P. pachydermus* utilized raffinose (weakly).

Assimilation of nitrogen sources: All species utilized ammonium phosphate, ammonium nitrate, ammonium acetate, ammonium sulphate, potassium nitrate, sodium nitrate, asparagine, urea, peptone, l-leucine, dl-methionine, dl-alanine, l-proline, l-aspartic acid and l-glutamic acid. Utilization of l-leucine was weak in *P. pachydermus*.

Under conditions approximate to Lodder and Kreger-van Rij's method, starch-like compounds were formed in the capsules and partly diffused into the media in all species.

Growth was good at 20° C, very slow at 25° C, no growth at 27° C; cultures died in 120 hours at 30° C.

No definite physiological difference between *P. inouyei* and *P. lactucae-debilis* was evident.

Spectrophotometric examinations of pigments were made to determine the possible relation between the pigments of these three species and of other reddish yeasts or yeast-like fungi. Pigment was extracted by the method of Ellinghausen and Pelczar (**1954**). One liter flasks containing 200 ml of malt agar were inoculated with the above three species. Cultures of *Taphrina wiesneri* (Rathay) Mix (*T. cerasi* (Fkl.) Sadeb.), isolated from *Prunus yedoensis* at Tokyo, and of *T. communis* (Sadeb.) Giesenh. furnished by Dr. A. J. Mix (Univ. of Kansas) were also used for comparative studies. All cultures were grown in stationary condition for 25 days at 20° C, after which the cells were removed by means of a glass needle and methanol extracts were obtained. The materials thus extracted were examined spectrophotometrically in a Beckman D U Spectrophotometer at a spectrum-band width of 5 mμ. The wavelength ranged from 320 to 700 mμ. Absorption peaks of the methanol extracts from the three species of *Protomyces* and the two of *Taphrina* were as follows:

Species	Absorption peak, in millimicrons
P. inouyei	320, 385
P. lactucae-debilis	320, 380
P. pachydermus	320, 385
T. wiesneri	320, 370, 390
T. communis	320, 380

The above results show that members of the two genera resemble each other closely as to pigmentation. Though colonies of these fungi are pinkish in color, none of them produced the carotenoid pigments which are characteristic of *Rhodotorula*.

INOCULATIONS

As inocula, heavy suspensions of cultivated cells were used. On April 16, inoculations by spraying with cells derived from blastospores of the three species were made on young plants of *Crepis japonica, Lactuca debilis* and *Taraxacum platycarpum.* Control plants were sprayed with distilled water. The plants used are known to be respectively susceptible to these species of *Protomyces.* Galls began to appear on leaves, pedicels and stems of *Crepis japonica* on May 2; on stems of *Lactuca debilis* on May 7; on leaves and stems of *Taraxacum platycarpum* on May 8; but no galls appeared on *Lactuca debilis* inoculated with *P. inouyei* nor on *Crepis japonica* inoculated with *P. lactucae-debilis.*

DISCUSSION

The position of the Protomycetaceae in taxonomy remains in doubt. Although many authors have discussed this family, ideas advanced are still speculations. Fitzpatrick (**1930**) placed the Protomycetaceae among the Phycomycetes and stated that they have no affinity with the Ascomycetes. Earlier Juel (**1902, 1921**) attempted to homologize the chlamydospore with the fertile cell of the ascogenous layer in the Exoascaceae (Taphrinaceae). Gäumann and Dodge (**1928**) followed this conception, and considered the Protomycetaceae and the Taphrinaceae as families of the order Taphrinales. Martin (**1950**) included the Protomycetaceae and the Taphrinaceae in the order Taphrinales, the chief character of which is a hyphal-cell becoming a chlamydospore or a single ascus.

Cytological investigations were also made in the present work, but the nuclei in *Protomyces* are by no means well defined and neither nucleolus nor chromatin, even in conjugated cells, could be differentiated in preparations stained by Heidenhain's method. Unfortunately the cells and nuclei are so small as compared with those of common yeasts that it was difficult to obtain factual data. Though the nuclear history is not yet clear, the present author believes that the Protomycetaceae belong to the lower Ascomycetes (following Martin's conception), and are closely related to *Taphrina* as the cultural investigations indicate. In one phase of their life cycle, the members of both genera are readily capable of yeast-like development by blastospores in artificial media. The parasitic, mycelial stage of both genera, involving sexuality, has resisted all attempts at culture. As stated above, cultural features of *Protomyces,* such as optimum temperature for growth, pigmentation of

the colonies, and size and shape of cells closely resemble those of *Taphrina*. Both *Protomyces* and *Taphrina* are oxidative. Three species of *Protomyces* assimilate ammonium acetate, and according to Mix (**1953**) this compound was refused by 79 cultures of Taphrina. No resting cells like those formed by *Taphrina* in culture have been observed in *Protomyces*. However, the results of this investigation point to an apparent interrelationship between *Protomyces* and *Taphrina*, and their close similarity is clearly evident. Macroscopically it is rather difficult to differentiate members of the two genera in culture.

Cultural stages of *Protomyces* must also be considered in relation to the yeasts. Phylogenetically the members of *Protomyces* may be considered as having originated from some members of the yeast group.

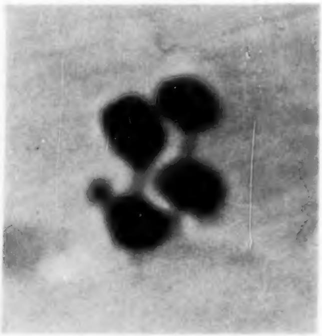

FIG. 3. *P. pachydermis*. Two pairs of conjugated cells stained with haematoxylin, × 2,000; first bud on lower left cell.

Mix (**1949**) indicated a close relation between the Taphrinales and the Endomycetales, and the present observations also clearly demonstrate a correlation between the Protomycetaceae and the Endomycetaceae or the Cryptococcaceae. Those yeasts which have never been known to produce ascospores in culture and are considered imperfect yeasts have been regarded by some authors as perfect yeasts which have lost their sexual abilities; they are now classified in the Cryptococcaceae. The genus *Protomyces* as represented by its saprophytic budding stage in culture would fall in the Cryptococcoideae including *Cryptococcus, Torulopsis, Candida,* etc. Formation of a starchlike substance by the budding state of *Protomyces* in culture is also commonly observed in *Cryptococcus*. Wickerham (**1952**) reports that some species of *Taphrina* produce starch in culture, and Mix (in personal correspondence)

states that all of his isolates (597, representing 39 species) of *Taphrina* form starch in culture under conditions of vigorous growth.

Torula infirmo-miniata Okunuki isolated by K. Okunuki (**1931**) from the air is assignable to the yeast stage of *Protomyces* from the following points. The maximum temperature for the growth of this species is 27°–28° C; it fails to grow at 29°–30° C. Growth is slow and the color of the colony is orange-pink or safrano-pink. These characters are similar to those of cultures of *Protomyces* and *Taphrina*. Unfortunately, this species is now lost. It is possible that other species of *Protomyces* may be found among budding fungi with low-temperature growth requirements.

As stated above, many species of *Protomyces* are known in nature. A variety of species should be studied by means of inoculation experiments and by cytological and physiological treatment. Such studies are now in progress.

SUMMARY

Eight cultures of *Protomyces* were obtained, distributed among species as follows: *P. inouyei* Hennings, 6; *P. lactucae-debilis* Sawada, 1; *P. pachydermus* Thuemen, 1. Their chlamydospores are able to germinate after a resting period of about seven months when placed in water at 20° C. Liberated spores from chlamydospores grow readily on artificial media and, like yeasts, multiply by blastospores without producing true mycelium. All species are oxidative and grow below 27° C. Three species of hosts have been shown by inoculation experiments to be susceptible to species of *Protomyces* and re-isolations have been made.

The genus *Protomyces* is considered from the point of view of its cultural stages to belong to the Taphrinales.

ACKNOWLEDGMENTS

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NAGAO INSTITUTE,
6-387, KITASHINAGAWA, SHINAGAWAKU, TOKYO, JAPAN

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OBSERVATIONS ON GYMNOASCACEAE. IV. A NEW SPECIES OF ARACHNIOTUS AND A RECONSIDERATION OF ARACHNIOTUS TRISPORUS[1]

Harold H. Kuehn

(with 32 figures)

The purpose of this paper is to present descriptions of two species of Gymnoascaceae, one a new species, the other a new combination, and to discuss and illustrate their developmental morphology. In the previous papers in this series (Kuehn **1955a**, **1955b**, **1956**) the writer presented the developmental morphology as well as the cultural and morphological characteristics of 4 species of *Myxotrichum*, 2 species of *Gymnoascus* and one species of *Eidamella*. The methods employed in the present study are similar to those described in the first paper of this series. The organisms were grown on various media in Petri dishes. Minute tufts of hyphae were removed and slides were prepared for examination with the microscope. For later stages, the peridium of the immature ascocarp, if such a structure existed, was removed under a dissecting microscope. By exerting pressure on the cover slip, the contents of the ascocarp were spread out on a slide, thereby making the croziers evident.

Van Tieghem (**1877**) was the first to describe a species now included in the genus *Arachniotus* when he published a brief description, with no illustrations, of the development of *Gymnoascus ruber*. This species later was transferred by Schroeter (**1893**) to a new genus, *Arachniotus*, as *A. ruber* (Van Tieghem) Schroeter. According to Van Tieghem, the gametangial initials were essentially the same as Baranetzky (**1872**) had described for *G. reessii*. Two branches from the same or different hyphae came into contact, spiralled about one another, and then ceased to elongate. From the base of the initials two branches grew up around the coil, but ceased growth and did not form a peridium. At the same time branches, which enclosed the coil for some distance, arose from

[1] The writer wishes to express his sincere appreciation to Dr. LeLand Shanor for his interest in the study, for helpful suggestions and for reading the revised manuscript. Grateful acknowledgment is made to Dr. C. W. Emmons and Dr. C. W. Hesseltine for supplying cultures of the fungi examined.

hyphae in the vicinity of the coil. These branches assumed a brick red color and developed into a peridium. The ascogonium was reported to "bud" to produce a branching system which eventually formed spherical asci.

Van Tieghem described the conidial apparatus as so strongly resembling a *Verticillium* that the species might be described later under that name. Upon examination of Van Tieghem's isolate, Eidam (**1886**) saw no such *Verticillium*-like conidial structure and he stated that Van Tieghem must have had contaminated cultures.

Eidam (**1886**) was unable to investigate the life history of *Arachniotus candidus* (Eidam) Schroeter, described by Eidam as a new species under the binomial *Gymnoascus candidus,* because his attempts to culture this species were unsuccessful. However, Dale (**1903**) did succeed in her study of the development of *A. candidus.* She described coiling initials, which developed after 3 weeks, usually from different parent hyphae, and which consisted of a central club-shaped antheridium surrounded in a symmetrical spiral by a thinner ascogonium. Fusion of the gametangia occurred after each had been delimited from the parent hypha by a septum. The ascogonium became divided into many cells from most of which ascogenous hyphae developed and rebranched often to form a dense cluster of hyphae. Further development was similar to that which Dale described in this same paper for *G. reessii.* Oidia, each one larger than an ascus, represented the imperfect stage.

DeLamater (**1937**), working with a species resembling *Arachniotus aureus* (Eidam) Schroeter, although not quite identical with it, reported crozier formation in the Gymnoascaceae for the first time, and he also discussed the morphological development. He stated that the two similar gametangia coiled about each other slightly, following which a septum separated each from its parent hypha. Fusion, which was seen in sectioned material, occurred either at this time or later. The antheridium elongated into a club-like cell while the ascogonium coiled about it and became septate, forming binucleate cells from which ascogenous hyphae arose. Paired nuclei from the parent ascogonial cell migrated into each ascogenous hypha. After many conjugate divisions of the nuclei, followed by cell wall formation separating daughter nuclei, croziers formed, the penultimate cells becoming asci. Fusion of the ultimate and antepenultimate cells resulted in a binucleate cell which often grew out to form a new crozier and, eventually, another ascus. Nuclear fusion in the ascus was followed by 3 nuclear divisions resulting in 8 nuclei. No vegetative hyphae were observed to grow out from the base of the coil, and the asci were nestled at random among the aerial hyphae with no

other special protective sterile cells about them that might correspond to a peridium.

Hotson (**1936**) described a new species, *Arachniotus trisporus,* characterized by possessing 3 types of spores, namely, conidia, chlamydospores, and ascospores. The conidia were in chains on bottle-shaped sterigmata which were formed either on short, simple, lateral branches scattered on the hyphae, or on penicillate structures. The gametangial initials resembled those of *A. candidus,* but in many instances an antheridium was reported to be absent. Ascogenous hyphae arose from cells of the ascogonium and branched often, with the apical cells eventually enlarging to form asci.

Rosenbaum (**1944**) presented more detailed observations on *A. trisporus.* According to her, the ascogonium was borne on the end of a long septate branch, while the antheridium formed on a short side branch which arose either from the same hypha or from a different one. The ascogonium usually grew to, and coiled about, an antheridium from another hypha, rather than one borne on the same hypha. Antheridial nuclei were not observed to move into the ascogonium, but the irregularly spaced antheridial nuclei were fewer in number after the two gametangia had come into contact. Also, the ascogonium at first had evenly spaced solitary nuclei, a condition which later was replaced by one in which there were evenly spaced pairs of nuclei. Ascogenous hyphae developed from the septate ascogonium. The primary croziers which were formed grew out to produce additional croziers. Eventually, asci were formed from the penultimate cells of croziers.

OBSERVATIONS

Arachniotus reticulatus sp. nov.[2]

Cleistotheciis plus minusve globosis, in agaro PDA flavis, in agaro Sabouraudii pallide brunneis, 126–883 μ diam. Peridii hyphis levibus vel tuberculatis, septatis, 1.4–2.8 μ diam., pallide flavis, hic illic brunneo vel fusco-brunneo tinctis. Ascis hyalinis, oblongis vel clavatis, 7–8.4 $\times$ 11.2–12.6 μ, octosporis. Ascosporis hyalinis, reticulatis, globosis, 2.8–3.2 μ diam. Hyphis sterilibus hyalinis, 0.8–2.1 μ diam. Oidiis hyalinis, 2–2.8 $\times$ 2.8–8.4 μ.

Cleistothecia roughly globose, yellow on PDA, light brown on Sabouraud's agar, 126–883 μ diam. Peridial hyphae smooth or tuberculate, septate, not cuticularized, 1.4–2.8 μ diam., light yellow, but often with segments tinged brown to dark brown. Asci hyaline, oblong to clavate, 7–8.4 $\times$ 11.2–12.6 μ, 8-spored, with the ascospores adhering in

[2] The writer acknowledges with appreciation the assistance of Dr. D. P. Rogers, New York Botanical Garden, in the preparation of the Latin diagnosis.

a ball after the ascus wall disappears. Ascospores spherical, hyaline, and reticulate with thick raised bands, so that the spores appear spiny in sectional view. Their diameter, including bands, is 2.8–3.2 μ; without the bands, 2.6–3 μ. Vegetative hyphae hyaline, 0.8–2.1 μ diam. Racquet mycelium also present. Imperfect spore phase represented by hyaline oidia which measure $2.0–2.8 \times 2.8–8.4\ \mu$.

Colonies on Sabouraud's agar are white, sparse, delicate in appearance, turn yellow in 15 days and brown 2–3 days later. Reverse of the colony is dull red-orange. Cleistothecia appear in 22–25 days as white tufts of hyphae, turn yellow and finally become light brown.

Colonies on PDA are white, sparse and delicate in appearance. Reverse of the colony assumes a pink tinge after approximately 6 days, although small black patches may appear later. Cleistothecia arise in 13 days as white tufts of hyphae at the periphery of the colony. Within a few days the ascocarps and the vegetative hyphae at the periphery of the colony form a yellow crust. This condition soon characterizes the entire colony, although scattered individual ascocarps later may become brown in color.

Several species in the Gymnoascaceae, as well as several of the imperfect dermatophytes, possess a distinctive odor which is typical of these fungi. *Arachniotus reticulatus* possesses such an odor, which may be described as being earthy, although it is not the fresh, earthy odor of certain Actinomycetes.

The isolate of *Arachniotus reticulatus* was received from C. W. Emmons labeled as *#5051*. It had been isolated from bat dung collected in Georgia.

This species is distinguished from all other species of *Arachniotus* in the possession of spherical, reticulate, ascospores. The other species with yellow ascocarps are *A. aureus* (Eidam) Schroeter and *A. citrinus* Massee and Salmon. *Arachniotus aureus* possesses fine hyphal spirals in the peridium and has spiny ascospores which are either spherical or elliptical but are of greater diameter than those of *A. reticulatus*. *Arachniotus citrinus* has smooth ascospores. *Arachniotus aureus* and *A. trachyspermus* Shear are the only species of the genus which possess spiny spores, and no other species are known to have reticulate spores. The specific epithet of *A. reticulatus* has been selected because of the diagnostic character of the ascospores.

The copulating branches in this species are similar in size and shape throughout the initial stages of formation. They arise from separate parent hyphae as lateral branches (FIG. 1), soon become longer and clavate, with a thick apex which gradually tapers to a narrow basal

portion (FIGS. 2–6). Usually these branches twist about one another only slightly, perhaps ½ turn (FIGS. 2–3), but there may be no coiling whatsoever (FIG. 4). As a rule, the two apices come into contact (FIGS. 2–5), but they may remain free (FIG. 6). Dissolution of the contiguous cell walls was not observed, nor was any other evidence of fusion noted. After each of the coiling organs is delimited from its parent hypha by a septum (FIGS. 3, 10), they become septate several times (FIGS. 2–6). The distal end of one branch coils about the apex of the other (FIGS. 5–7). Since this coiling branch is the one from which ascogenous hyphae are produced later, it is designated as the ascogonium, while the other branch is the antheridium. Many septa form in the coiling portion of the ascogonium (FIG. 7) with ascogenous hyphae originating from most of the cells thus formed. The ascogenous hyphae usually remain short and produce croziers immediately (FIGS. 8, 10). Asci do not form from these original croziers, but instead the penultimate cell grows out to form another crozier (FIG. 15). The cell formed by fusion of the antepenultimate and ultimate cells of the crozier often produces a short hypha which recurves to form a crozier. In this manner the ascogenous hyphae branch repeatedly to produce a dense cluster. Eventually, asci develop from the penultimate cells of croziers which form later.

Peridial hyphae arise as branches of the parent hyphae near the coil (FIGS. 3, 10). Vegetative hyphae in the vicinity of the initials also enter into formation of the peridium and branch repeatedly to produce a peridium which delimits a definite cleistothecium. Some peridial hyphae remain thin, resembling the vegetative hyphae in this respect, but the majority of the peridial hyphae become tuberculate.

BYSSOCHLAMYS NIVEA Westling, Svensk Bot. Tidskr. II. **2**: 125. 1909.

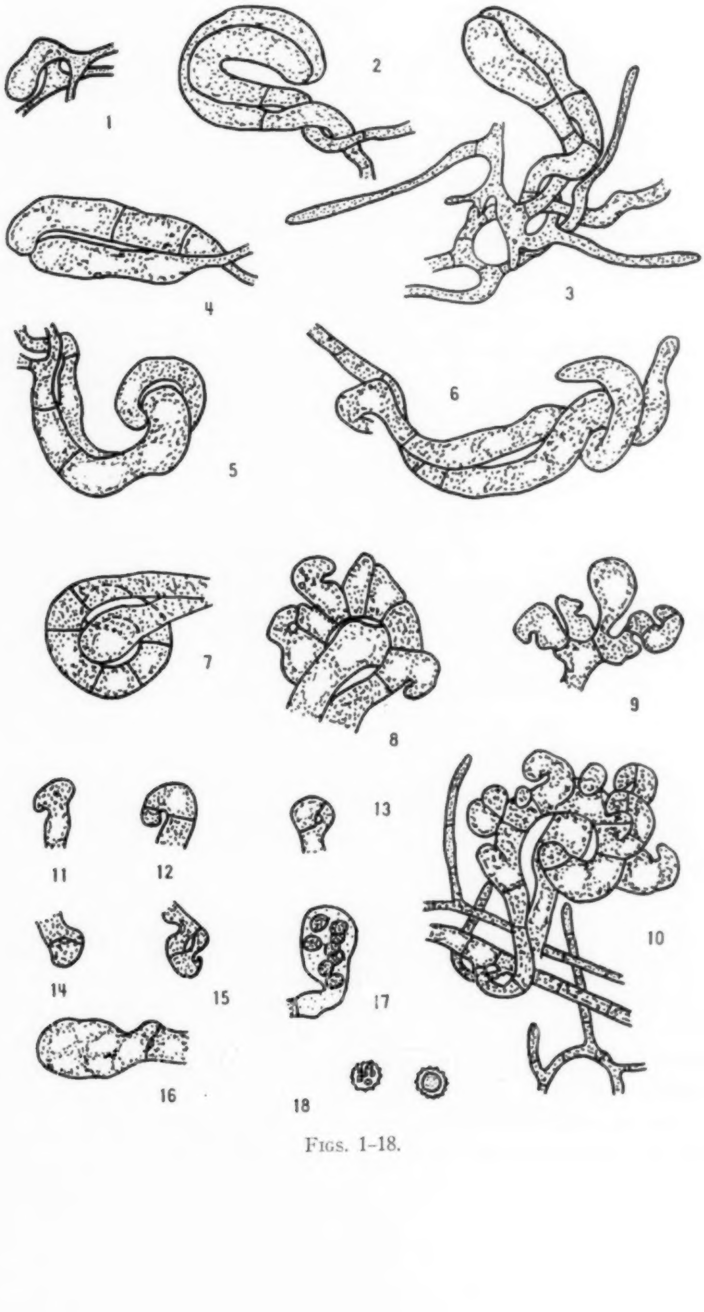
Arachniotus trisporus Hotson, Mycologia **28**: 500. 1936.

Gymnoascus sudans Vailionis, Didz. Univ. Mat. Gamt. **11**: 115. 1936.

Byssochlamys trisporus Cain, Can. Jour. Bot. **34**: 140. 1956.

Hotson (**1936**) briefly described the development of the ascocarp of *Arachniotus trisporus*. His description differs from that of Rosenbaum (**1944**) and the author in that Hotson reported an antheridium was not always present, and that asci were formed by swelling of the apices of ascogonial branches.

The work of Rosenbaum already has been reviewed. Her work has been confirmed during the course of the present investigation. However,



FIGS. 1-18.

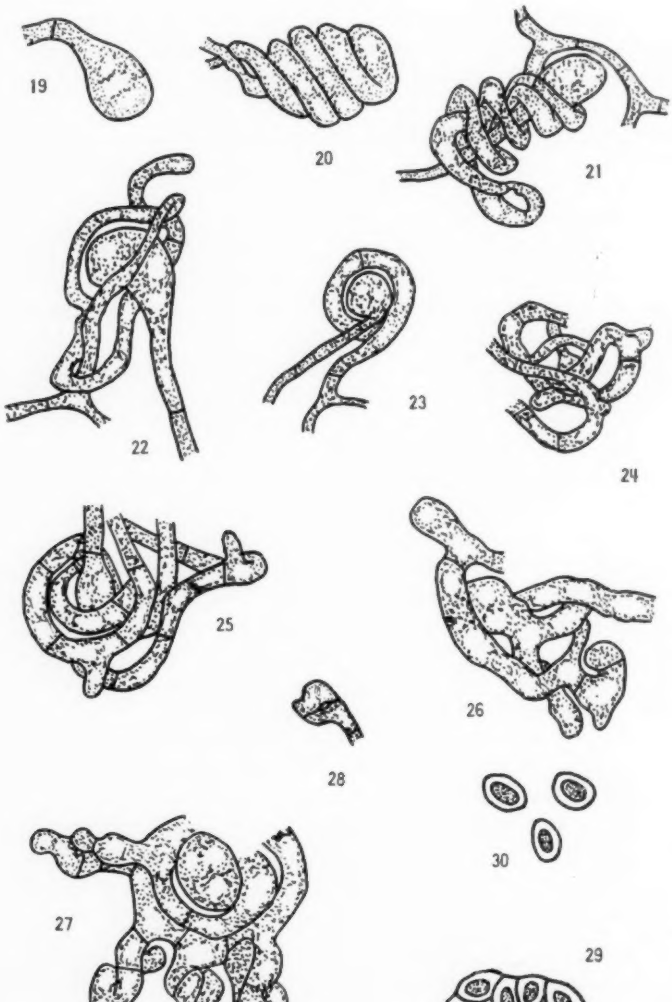
since certain additional information has been obtained, it is desirable to present here a complete description of the species, including the cultural characteristics. Four isolates, including a subculture of Hotson's, were studied.

Colonies on Sabouraud's agar grow rapidly. At first they possess a sparse, mucoraceous type of white mycelium which becomes cream colored as the asci mature. Groups of asci appear in 4–5 days as white tufts on the colony. These groups of asci become cream colored, and are so numerous that they cover the entire surface of the colony. Mature asci are found within 7 days. Reverse of the colony is yellow. An exudate is produced which consists of small colorless droplets which later collect to form larger yellow droplets.

Cleistothecia are absent. The asci lie exposed in clusters on the hyphae with or without loosely aggregated vegetative hyphae about them. The groups of asci are approximately spherical, mostly from 37.8–151.2 μ diam., although the individual groups of asci may merge into larger clusters of indefinite limitations which appear to be discrete cleistothecia. These clusters, however, lack a definite peridium and are not ascocarps. Extremely small groups containing 4 to 8 asci may be present also. Asci are hyaline, obovate, 8.1–8.5 $\times$ 11.2–12.6 μ, 8-spored. The ascus walls are ephemeral, with the spores adhering in balls with dimensions of 8.2–8.5 $\times$ 11–11.3 μ. Ascospores are hyaline, ovoid, smooth, 2.8–4 $\times$ 3.4–5.6 μ. Vegetative hyphae are of two types: thin, 1.2–1.5 μ diam., and thick, 4.2–8.4 μ diam. Racquet mycelium is also present. Chlamydospores are borne singly on undifferentiated lateral branches of varying lengths. Mature chlamydospores are 6.3–7.2 $\times$ 7–8.2 μ, ovoid to pyriform, with hyaline to light yellow walls which are slightly roughened. Conidia are in chains on sterigmata which are borne singly on short branches, or on penicillate structures. The conidia are hyaline to light yellow, smooth, ellipsoid, and measure 3.7–4.4 $\times$ 4.5–5.3 μ.

Four strains of this species were examined. One was isolated by R. K. Benjamin from sorghum silage at the University of Illinois.

FIGS. 1–18. *Arachniotus reticulatus.* 1. Young gametangial initials arising from different hyphae. 2–6. Various types of orientation of gametangial branches. Fig. 5 shows the ascogonium coiling about the apex of the antheridium. 7. A septate ascogonium coiling about the apex of an antheridium. 8. Short ascogenous hyphae forming croziers which grow from most of the cells of the ascogonium. 9. Various stages in crozier and ascus formation at the apex of an ascogenous hypha. 10. An older coil showing ascogenous hyphae with croziers. 11–14. Stages in crozier formation. 15. The penultimate cell of a crozier continuing growth as an ascogenous hypha. 16. A young ascus. 17. A mature ascus. 18. Mature ascospores, surface and sectional views. All figures $\times$ 1300.

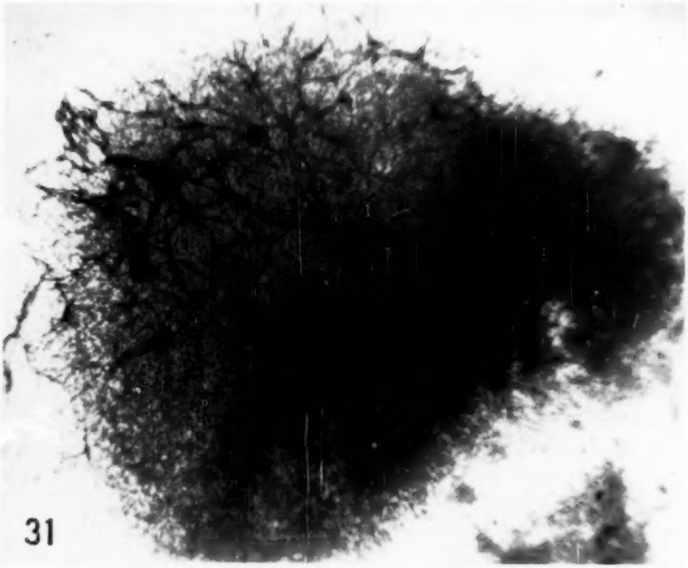


FIGS. 19–30. *Byssochlamys nivea*. 19. An antheridium separated by a septum from the branch on which it arises. 20. An ascogonium coiling about the central antheridium. 21. A several-septate ascogonium coiling about an antheridium from another parent hypha. 22. An ascogonium forming an extremely loose coil about

Another strain, NRRL *1678* (*#5170.1* in the Thom collection), was obtained from C. W. Hesseltine. It was found growing on submerged coal, Norfolk, Virginia. Both of these strains were unidentified. A subculture of Hotson's isolate was obtained from the Centraalbureau voor Schimmelcultures as was a culture of *Byssochlamys nivea* Westling.

The gametangial initials arise simultaneously and terminally on separate lateral branches which originate from the same parent hypha. However, ascogonia tend to unite with antheridia from different parent hyphae. The antheridium is formed as a club-shaped apical swelling on a short lateral branch (FIG. 19). A branch from a neighboring hypha grows toward the antheridium and coils about it 5–6 times in a tight (FIG. 20) or loose (FIG. 21) spiral, or in a rather indefinite manner which may suggest an extremely loose spiral (FIG. 22). Before the coiling has progressed very far each gametangium is separated by a septum from the branch on which it is borne. Further septation does not occur in the antheridium. The contiguous walls which separate a pair of gametangia were not seen to dissolve to effect a sexual fusion. Septa divide the ascogonium into several cells, following which some of these cells bulge out to become ascogenous hyphae (FIGS. 24, 25) that remain short and soon form croziers (FIG. 26). From the penultimate cell of each of these initial croziers the ascogenous hypha grows forth to produce a new crozier (FIG. 27). This process continues until the penultimate cells of croziers swell to form asci. The cell which results from fusion of the ultimate and antepenultimate cells grows out to form a crozier. In this manner each ascogenous hypha rebranches often, so that many asci arise from a single pair of gametangia. No sterile appendages grow forth from the base of the coil. Each cluster of asci is without any trace of a peridium, although inadvertently the vegetative hyphae may surround one to many groups of asci in such a manner as to give the appearance of a cleistothecium. However, when examined under the microscope, these are seen to be not definite entities, but only many individual groups of asci located at random in loose tufts of hyphae. Thus, since cleistothecia with a peridium are lacking, this fungus would seem to belong in *Byssochlamys* rather than in *Arachniotus*.

the antheridium. 23. A pair of coiling initials as seen from below. 24, 25. Origin of ascogenous hyphae from a septate ascogonium. 26. A very loose coil showing ascogenous hyphae and one crozier. 27. An older coil as seen from above. The ascogenous hyphae have formed croziers from the penultimate cells of the primary croziers. 28. A crozier showing fusion of the ultimate and antepenultimate cells. 29. Mature ascus containing ascospores. 30. Mature ascospores. All figures × 1300.



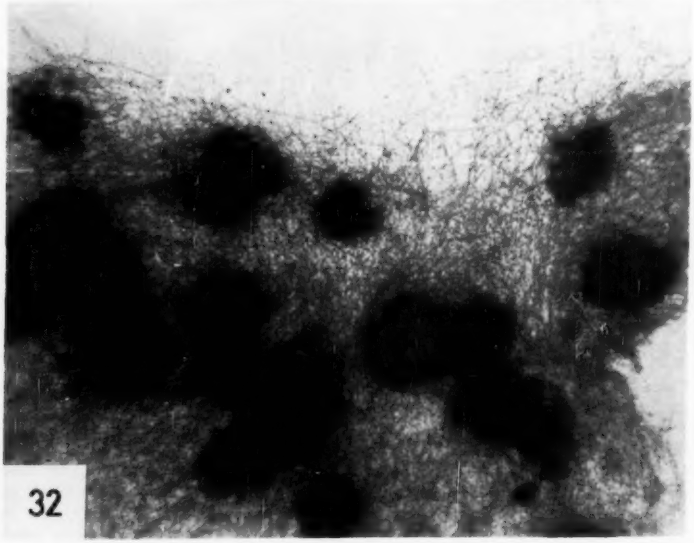


FIG. 31. Cleistothecium of *Arachniotus reticulatus*, × 117.

FIG. 32. *Byssochlamys nivea*, showing the individual groups of asci scattered about in the vegetative hyphae, × 103.

DISCUSSION

The family Gymnoascaceae is generally recognized as intermediate between the Endomycetaceae of the subclass Hemiascomyceteae and the Eurotiaceae of the subclass Euascomyceteae. In the development of ascogenous hyphae and croziers, and in the possession of a peridium in the majority of species, the Gymnoascaceae resemble the Eurotiaceae. The possible evolutionary development of a peridium may be traced from the condition found in the Endomycetaceae through a series of increasing peridial complexity in the Gymnoascaceae to the more highly organized type of peridium characteristic of the Eurotiaceae.

Byssochlamys is a genus which is somewhat intermediate between the Endomycetaceae and the Gymnoascaceae, and it often is placed in the former. This genus resembles the Endomycetaceae only in the lack of a peridium. Generally, two species have been included in the genus *Byssochlamys*, *B. nivea* Westling (**1909**) and *B. fulva* Olliver and Smith (**1933**). Recently, a third species, *B. trisporus* (Hotson) Cain (**1956**), was transferred from *Arachniotus* to *Byssochlamys*. These species possess fundamental characteristics which indicate their relationship to the Gymnoascaceae. These are as follows: 1, they produce conidial structures of a penicillate type; 2, the gametangia are of a gymnoascaceous type; and 3, they produce ascogenous hyphae. Emmons (**1935**), working with *Penicillium* species, included a short note to the effect that the asci in *Byssochlamys fulva* were produced by means of croziers. His investigation of the development of *B. fulva* was not intended to be complete. Recently Benjamin (**1956**) illustrated croziers for *B. nivea*. Neither ascogenous hyphae nor croziers are encountered in the Endomycetaceae. Therefore, *Byssochlamys* may be considered to be the most primitive genus of the Gymnoascaceae.

Cain (**1956**) was the first investigator to realize that *A. trisporus* and *Byssochlamys* were congeneric and proposed the new combination *B. trisporus* (Hotson) Cain. However, Benjamin (**1956**) reported that *A. trisporus* should be considered to be a synonym of *B. nivea* Westling. Although the present writer initially considered *B. trisporus* to be a distinct species (**1954**), reexamination of the available strains forces him to agree with Benjamin.

According to Dale (**1903**), in *Arachniotus candidus* the asci were completely without investment, and were aggregated into dense clusters. Each cluster was produced from a single pair of gametangia. Certain other fungi that have been described as species of *Arachniotus* have been reported to lack completely a peridium surrounding the asci. For example, DeLamater (**1937**), working with an isolate very similar to

Arachniotus aureus, reported that the asci were nestled at random among the aerial hyphae with no structure present which might correspond to a peridium. *Arachniotus citrinus* Massee and Salmon was reisolated by West and Ajello (**1956**). They did not attempt to elucidate the developmental morphology, except to note the formation of gametangial initials. However, they did report that the asci developed from the coiling initials and that a peridium was lacking completely. It would seem now that all such fungi as *A. candidus, A. citrinus* and DeLamater's *A. aureus* (?) belong more properly in the genus *Byssochlamys* and probably should be transferred to that genus. A future paper will present the results of an investigation of *A. citrinus.*

SUMMARY

1. Diagnostic characteristics are provided for a new species, *Arachniotus reticulatus,* and also for *Byssochlamys nivea.*

2. The morphological development of these two species is discussed and illustrated. The formation of ascogenous hyphae and croziers precedes the production of asci in both species. This agrees with the findings of Rosenbaum for *Arachniotus trisporus* Hotson and of Benjamin for *Byssochlamys nivea* Westling.

3. *Arachniotus trisporus* Hotson was found to lack a true peridium. Therefore, this species falls within the generic limits of *Byssochlamys* as was previously recognized by Benjamin and by Cain. However, the results obtained here verify the position taken by Benjamin in placing *A. trisporus* Hotson in synonomy with *B. nivea* Westling.

4. The position of the genus *Byssochlamys* within the family Gymnoascaceae is discussed.

SPACE BIOLOGY BRANCH,
AERO MEDICAL FIELD LABORATORY,
HOLLOMAN AFB, NEW MEXICO

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A FURTHER STUDY OF KARLING'S KERATINOPHILIC ORGANISM [1]

FREDERICK M. ROTHWELL [2]

(WITH 2 FIGURES)

In 1954 Karling described an unusual microorganism from soil cultures which had been baited with keratinized tissues such as human hair, snake skin and feathers. He did not name this organism but assigned it provisionally to the family Actinoplanaceae of the Actinomycetales. The author undertook a further study of this species with the object of isolating and growing it in pure culture, determining its nutritional requirements and successive developmental stages, and the number and position of the flagella on its spores. Accordingly, the present contribution concerns certain aspects of this study as well as a description of what appears a different but related species.

The material for this study was secured from soil which was flooded with animal charcoal water and baited with human hair. The soil samples were collected in the vicinity of the Purdue University campus as well as in Pennsylvania, New Jersey and New York. Karling's organism was present in one or more samples from each of these localities, which shows that it is widely distributed geographically. In attempting to isolate this organism in pure culture, the infected hair first was thoroughly washed several times in distilled water and then placed in a drop of sterile charcoal water on a glass slide. By scraping the hair with a sterile razor blade, a great number of sporangia were loosened into the water. The hair was then removed, and a portion of the water was pipetted onto a sterile cover glass. With the de Fonbrune micromanipulator single sporangia were isolated and transferred to petri dishes with Krainsky's asparagine-glucose agar, nitrate-sucrose agar, ammonium sulphate-starch agar, tryptic digest of casein-yeast

[1] This study was done under the guidance of Dr. J. S. Karling while the author was a Public Health Service Postdoctorate Research Fellow of the National Cancer Institute. Soil samples were received from Dr. Albert Schatz, National Agricultural College, Bucks County, Pennsylvania; Dr. S. O. Thomas, Lederle Laboratories, Pearl River, N. Y., and Mr. Joseph Riedhart and Mr. Joseph Becker, Purdue University.

[2] Present address: Buckman Laboratories, Inc., Memphis, Tenn.

extract-fatty acid agar, Czapek's agar and potato dextrose agar. In addition, a serial dilution method was used. Several thoroughly washed strands of hair were placed in the first dilution bottle and approximately 30 minutes later, when the sporangia were actively discharging spores, the dilution procedure was begun. One ml aliquots were taken from each of the five bottles used and plated out on the nutrient agars listed above. Another method was tried by which the infected hair was pulled through a 0.5% agar suspension several times and then placed upon the nutrient agar plates listed above.

Although the majority of contaminants were eliminated by one or more of the methods listed above, a pure culture of the keratinophilic organism was never obtained.

Microscopically, the sporangia and spores were studied with the ordinary light microscope, electron microscope, and in dark field illumination. Although dark field illumination is usually fruitful in studying the flagella of bacteria it failed to reveal the flagella on the minute spores of Karling's organism. However, it did show the presence of one to four refractive globules in the spores which are similar to those reported by Couch (**1954**) in spores of *Actinoplanes*. In electron microscope images, on the other hand, the flagella and their point of attachment are clearly visible. As shown in FIGS. 1, b and 1, d–f, the spore is oblong or rod-shaped and has a single polar flagellum. In FIG. 1, d the spore is tapered at the point of attachment of the flagellum, but this is not evident in the isolated spores shown in FIGS. 1, e and 1, f. The latter appearance is the more common and is considered to be typical. FIG. 1, a, is a light microscope picture of a typical sporangium with its stalk. This picture is slightly out of focus because it is impossible to get the globular sporangium and the stalk in the same focal plane. A portion of a smashed sporangium photographed by the electron microscope is shown in FIG. 1, c, and in this picture the spores have the same linear arrangement as in living sporangia.

The morphology of the spore of this microorganism is quite different from the spores described by Couch for the genus *Actinoplanes* (**1955**). First, the spores shown here have a single polar flagellum instead of several polar flagella; secondly, they are rod-shaped and not globose or sub-globose; and third, the spores within the sporangium are never in coils, but in straight chains, or sometimes irregular, due to breaking up of the spore chains before dehiscence of the sporangium. Karling noted that the motility of these cells or spores is typical of bacterial cells and quite unlike that of fungus zoospores. Also, in *Actinoplanes*, Couch (**1954**) stated that from stained cells and electron microscopy studies,

the flagella on the spores appear to be more like those found in certain bacteria than those of the lower fungi. The electron microscope pictures of the spores shown in FIG. 1 further substantiate these views. At any rate, the flagellum of these spores is obviously unlike those of certain of the lower Phycomycetes shown by Manton *et al.* (**1952**).

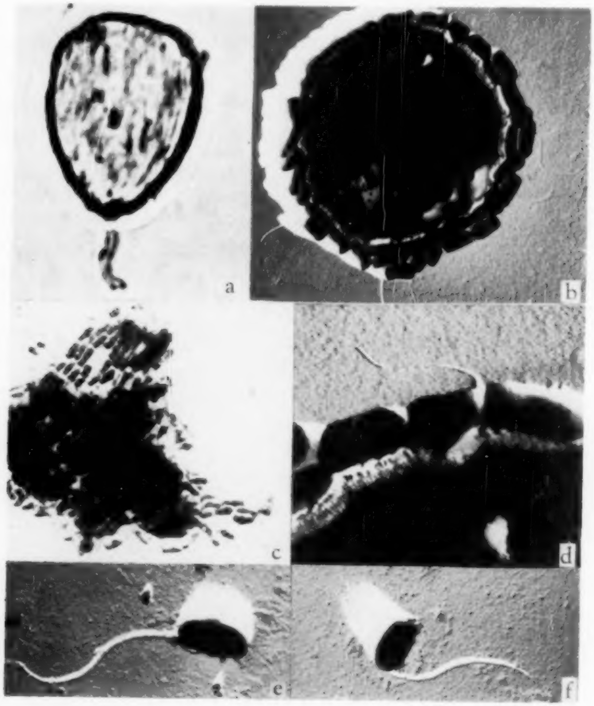

FIG. 1. a. Light microscope picture. b-f. Electron micrographs taken with the Philips electron microscope. The material was air dried on a collodion membrane and lightly shadowed at a 30° angle with pure chromium. Pictures were taken at 60 KV. a. Globular sporangium and stalk, × 2000. b. Cluster of rod-shaped spores, × 5600. c. Part of a sporangium showing the linear arrangement of spores, ×2500. d–f. View of rod-shaped spores showing attachment of single polar flagellum, d, × 30,000; e, f, × 12,500.

During this study of Karling's organism another related species was isolated from soil in a wooded area near Highway 52 which bypasses Lafayette, Indiana. It differs primarily by the cylindrical shape of the sporangia and the length of the stalks, and these differences are quite

conspicuous when both species occur on the same hair filament. The first evidence of growth of this microorganism on the hair in water is the appearance of fine, hypha-like strands (FIG. 2, a). These continue to elongate and extend outward from the hair, and after 3 to 4 days the tips of these strands begin to enlarge. These are the spo-

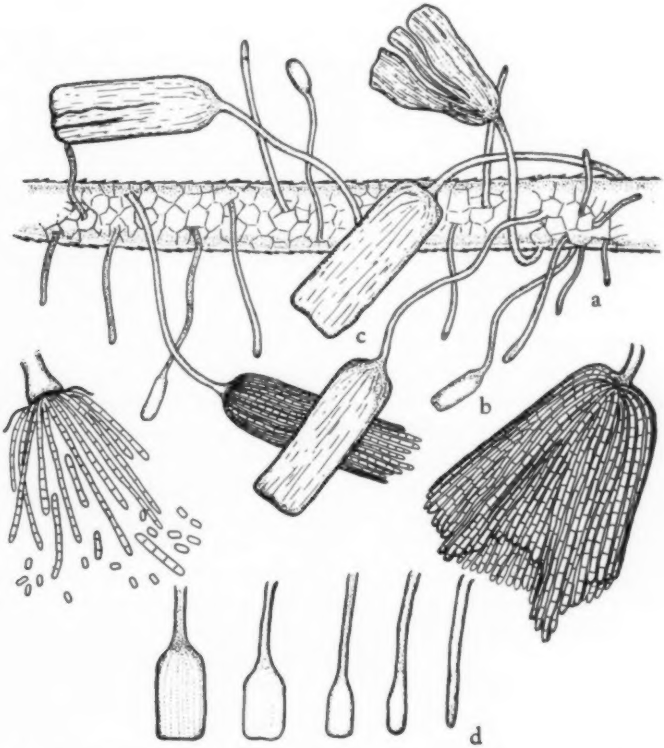

FIG. 2. a. Fine, hyphae-like strands, representing early stages of growth. b. Young stage in sporangium development. c. Mature sporangia in various stages of development. d. Early stages of sporangium formation.

rangium initials and soon develop into mature sporangia (FIGS. 2, b; 2, d). The cylindrical sporangia are 15–28.5 μ long by 4.5–8.25 μ in diameter (FIG. 2, c). The long, slender stalks, which may vary from 40–120 μ in length and 0.8–2 μ in diameter, become entangled quite readily, and it is extremely difficult to trace the stalk to its origin on

the hair. The rod-shaped spores are 0.75–1 μ in diameter by 1–2 μ long, motile, with a single polar flagellum. The flagellum is comparatively short, measuring only 2–2.5 times the length of the spore, and appears to be uniform in diameter throughout its length. Under dark field illumination, from one to four refractive globules per spore are visible as in the spores of the previous species.

Up to date, neither germination of the spores nor the presence of a mycelium has been found for this and Karling's species. Recently, however, Gaertner (**1955**) reported the presence of an intra- and extramatrical mycelium for a keratinophilic microorganism which closely resembles and appears to be synonymous with the species described by Karling (**1954**).

SUMMARY

Electron microscope pictures show a single polar flagellum on the spores of Karling's keratinophilic organism. All attempts to isolate and grow it in pure culture on synthetic media were unsuccessful.

What appears to be a different but related species was isolated from soil samples baited with human hair. It is characterized by cylindrical sporangia borne on long, slender stalks. The sporangia contain rod-shaped spores in long filaments. These spores are motile, with a single polar flagellum.

DEPARTMENT OF BIOLOGICAL SCIENCES,
PURDUE UNIVERSITY,
LAFAYETTE, INDIANA

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SYNCHYTRIUM DECIPIENS AND SIMILAR SPECIES

John S. Karling

In 1872 Peck described a fungus on *Amphicarpaea* (*monoica*) *bracteata* in New York which he named *Uredo aecidioides.* This binomial, however, had been used previously by De Candolle (**1805**) for a rust which Schroeter later (**1885**) transferred to *Melampsora.* Accordingly, the name *U. aecidioides* was preempted by De Candolle's early use of it for another fungus. Farlow (**1878**) found Peck's species on the same host near Boston and described it as *Synchytrium fulgens* var. *decipiens* because at that time he was unable to distinguish it specifically from Schroeter's *S. fulgens* on *Oenothera biennis.* In **1885** he established it as separate species, *S. decipiens,* and pointed out that it was the same fungus that Peck had described as *U. aecidioides.* In December, 1889, Lagerheim collected a species of *Synchytrium* on *Psoralea mutisii* near Quito, Ecuador, which he and Patouillard (**1891**) believed to be identical with Peck's fungus. Therefore, apparently unfamiliar with De Candolle's previous use of *U. aecidioides,* they named their fungus *S. aecidioides* and listed Peck's binomial as well as Thümen's *Uredo peckii* and Farlow's *S. fulgens* var. *decipiens* and *S. decipiens* as synonyms. They did not describe their fungus and apparently assumed from superficial appearances that it was identical with Peck's fungus. Nevertheless they were the first to use the name *aecidioides* in conjunction with *Synchytrium,* and in this manner their names became erroneously associated with Peck's species.

Since that time there has been considerable doubt about the identity of the Ecuadorian species with *U. aecidioides* Peck or *S. decipiens* Farl., and this together with Patouillard and Lagerheim's error has led to some nomenclatural confusion. I (**1954**) was unaware of De Candolle's previous use of *U. aecidioides* and referred to Peck's fungus as *S. aecidioides.* Tobler (**1912**) excluded Patouillard and Lagerheim's fungus from the genus on the ground that the specimens of it in the Berlin herbarium consisted of membrane-free sori (?) which she reported are

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similar to those of other *Synchytrium* species on *Psophocarpus* and *Vigna*. I also questioned (**1954**) its identity with Peck's *U. aecidioides*, because my cross inoculations indicated that the latter will not parasitize other hosts besides *A. bracteata* and its variety *comosa*. I suggested that the parasite on *Psoralea mutisii* might be a different species or possibly a biological race of Peck's fungus. Recently, through the courtesy of Dr. Donald P. Rogers of the New York Botanical Garden, I had the opportunity of studying the material collected by Lagerheim in 1889, and this study has proven conclusively that his species is not identical with Peck's fungus. Fixed and stained sections show that it is a long-cycled species which develops prosori, sori, sporangia and resting spores, and for this reason it must be removed from the subgenus *Woroninella* to which Peck's species belongs. Seven other similarly long-cycled species have been described on species of the Leguminosae, but the one on *Psoralea mutisii* differs from these in several respects. For this reason it is diagnosed as a new species and named in honor of its collector, who was an ardent student of the chytrids.

Synchytrium lagerheimii sp. nov.

S. aecidioides Patouillard & Lagerheim (non *U. aecidioides* Peck). 1891. Bull. Soc. Mycol. France **7**: 168.

Prosoris ovalibus, 130–150 × 170–200 μ, vel subsphaericis, 98–195 μ; soris ovalibus, 168–418 × 180–624 μ, vel hemisphaericis, 115–140 × 160–180 μ, parietibus laevis, 2–3 μ crasso; sporangiis numerosis, polyhedris 24–36 μ; sporis perdurantibus ovalibus, 115–140 × 160–190 μ, vel subsphaericis, 120–170 μ, parietibus laevibus, 4–5 μ crasso; germinatione ignoto.

Prosori solitary, lying in apex of host cell when empty and collapsed, ovoid, 130–150 × 170–200 μ, or subspherical, 98–195 μ. Sori ovoid, 168–418 × 180–624 μ, with a wall 2–3 μ thick and pale yellowish-orange content; plug between empty prosorus and sorus 5–7 μ thick and 8–12 μ diam. Sporangia 60 to almost 1000 per sorus, polyhedral, 24–36 μ, with fairly thick hyaline walls and yellowish-orange content. Zoospores unknown. Resting spores solitary, not filling host cell completely, ovoid, 115–140 × 160–190 μ, or subspherical, 120–170 μ, with a smooth wall, 4–5 μ thick; enveloping residue very sparse or lacking; germination unknown.

Compositely dihomeogallic, galls separate and scattered or sometimes crowded and confluent, usually protruding fairly conspicuously. Sporangial galls yellowish, broadly mound-shaped or hemispherical, 214–302 μ high by 220–680 μ broad, with a broad apical pore up to 30 μ diam., cupulate after dehiscing, sheath 3–6 cells thick. Resting-

spore galls broadly mound-shaped, 203–240 μ high by 312–360 μ broad; sheath 3–5 cells thick.

On leaves and petioles of *Psoralea mutisii,* near Quito, Ecuador. (Leg. Lagerheim, 12–1889.)

The dehisced sporangial galls of this species are distinctly cupulate and resemble those of members of the subgenus *Woroninella,* so that it is not surprising that Lagerheim mistook it for Peck's fungus. However, the sheath is usually much thicker, and there is no evidence of lysis of the inner sheath cells as is reported to occur in *S. decipiens.* Most of the sporangial galls are quite large, and when these are confluent and have dehisced they may resemble those of *S. shuteriae.* In very large galls the sori may be unusually large also and seem to contain up to 1000 or more sporangia, which are larger than those of *S. decipiens.* So far no stages of sorus development from the prosorus have been observed, but in intact galls an empty and collapsed vesicle lies above the sorus in the infected cell. I interpret this as the remnants of a prosorus. More important, a plug of densely stainable material connects the empty vesicle to the sorus, and I (**1955a**, **b**, **c**) have found such plugs in all species where the initial cell has functioned as a prosorus. In view of this as well as its long cycle of development, *S. lagerheimii* is placed temporarily in the subgenus *Microsynchytrium,* but its final inclusion there depends on the manner in which the resting spores germinate.

As noted earlier, seven other similarly long-cycled species, *S. cookii, S. crustaceum, S. zorniae, S. cassiae, S. marginale, S. parksii,* and *Synchytrium* sp., have been described on other species of the Leguminosae. The first four of these occur in India (Lingappa, **1953**, **1955**) and the last three in the United States (Karling, **1955b**, **1956**). *Synchytrium zorniae* and *S. cassiae* are diheterogallic in relation to their hosts, while the other five are compositely dihomeogallic like *S. lagerheimii.* The sporangial galls of all species, however, are cupulate after dehiscing, and this is characteristic of all species known to occur on the Leguminosae, regardless of whether they are long- or short-cycled. Accordingly, the presence of cupulate galls is not a distinctive criterion for differentiating these species. Nevertheless, *S. lagerheimii* differs from the other long-cycled species by its larger prosori, sori and resting spores and by a greater number of sporangia per sorus. Furthermore, its sorus is formed beneath the prosorus, whereas in *S. marginale, S. parksii* and *Synchytrium* sp. it is formed above. So far, I have found the relative positions of the prosorus and sorus to be fairly constant and specific.

UNIDENTIFIED MEMBERS OF WORONINELLA

In addition to *S. decipiens,* most herbaria in the United States contain specimens which are labeled *S. aecidioides, S. fulgens* var. *decipiens, S. decipiens,* or *Synchytrium* sp. and occur on other leguminous hosts besides *Amphicarpaea bracteata.* In light of my cross inoculation experiments, which indicate that *S. decipiens* on *A. bracteata* from Indiana has a very limited host range, I have doubted that these specimens are identical to Peck's fungus. Accordingly, I have studied them also to determine their identity and relationships as far as is possible from herbarium material. So far no prosori and resting spores have been found, and all species appear to develop only sori and sporangia in composite galls which become cupulate and aecidium-like upon dehiscence. Apparently, they are short-cycled and belong in the subgenus *Woroninella.* However, until they are more fully known and their host ranges have been determined it is not worth while to give them specific names. Nevertheless, a description of them is presented herewith to focus attention on their occurrence and to stimulate further study of them by collectors.

SYNCHYTRIUM sp. on *Apios* (*tuberosa*) *americana.* Boone Co., West Va., U.S.A. (Leg. B. T. Galloway, 6–1887; spec. in herb. Univ. Calif., Berkeley.)

Sori oval to subspherical, 98–300 μ diam. Sporangia up to several hundred or more per large sorus, polyhedral, 8–16 μ in greatest diam. with very thin, hyaline walls. Zoospores unknown.

Compositely monogallic, sporangial galls on leaves and petioles, usually scattered or crowded and confluent; somewhat dome- or mound-shaped or hemispherical, 110–180 μ high by 128–360 μ broad, cupulate after dehiscing; sheath 2–6 cells thick.

In the material at hand this species resembles Peck's fungus in general appearance and by the size of its sporangia.

SYNCHYTRIUM sp. on *Eriosema grandiflora,* Cuernavaca, Mexico. (Leg. Holway, 26–8–1898; spec. in Arthur Herb., Purdue Univ.)

Sori 110–206 μ diam. Sporangia numerous to several hundred per sorus, polyhedral, 15–20 × 24–30 μ, oval, oblong, elongate and usually angular with hyaline walls and faintly yellow content. Zoospores unknown.

Compositely monogallic, sporangial galls most abundant on lower surface of leaves, scattered and separate or aggregated and confluent, cupulate and aecidium-like after dehiscing, 160–280 μ diam. on leaves; oblong to elongate on midrib and veins.

Several fairly similar species have been collected on *Eriosema* in the tropics, and one collected by A. S. Muller, 7–27–1950, on *E. diffusans* (Spec. No. *53031*, Arthur Herb. Purdue Univ.) at Santa Clara, Honduras, seems to be identical with the species collected by Holway. In Muller's material, the sporangial galls are abundant on the lower surface of the leaves, where they are usually crowded and confluent. The galls are broadly mound-shaped, 187–230 μ high by 370–580 μ broad, and become cupulate after dehiscing. The sporangia are polyhedral, 14.4–24 μ in greatest diameter, with thin hyaline walls.

Another fungus was collected by P. Dusen, 1–18–1909, on *E. glabratum* at Ponta Grossa, Parana, Brazil. In this species the sori are subspherical to oval, 276–310 × 312–468 μ, and bear several hundred sporangia, which are polyhedral, 16–30 μ in greatest diameter, with thin hyaline walls and hyaline content. The exceptionally large sporangia may be 36–43 μ in diameter. The induced galls are yellowish-brown and usually scattered and separate on the underside of the leaf, but sometimes they are crowded and confluent. They are mound-shaped, hemispherical or subspherical, 312–416 μ high by 364–572 μ broad, and may protrude on the opposite side of the leaf also. The sheath is 4 to 7 cells thick, and the inner sheath cells are filled with a dense resin-like substance.

The sori and sporangia of this Brazilian species are considerably larger than those of the ones described previously on *Eriosema,* and it may prove to be another species.

Still another *Synchytrium* species was collected by E. Mayor, 8–15–1910, on *Eriosema* sp. in the Department of Antioquia in southern Columbia, at an altitude of 1700 meters. (Spec. in Farlow Crypt. Herb., Harvard Univ.) It causes large, composite, yellow, separate or crowded and confluent mound-shaped galls, 182–270 μ high by 250–700 μ broad on the underside of the leaves, which are surrounded by a reddish-brown area. The sheath is 3 to 5 cells thick, and the inner sheath cells are filled with a resin-like substance. The sorus is broadly fusiform, 130–208 × 357–650 μ, or ovoid, 208–220 × 218–234 μ, and bears several hundred polyhedral sporangia, 18–30 μ in greatest diameter, with hyaline walls and yellow content.

Most of the sori in Mayor's material are immature and uninucleate, but in a few of them sporangia were present. Although the sori may be larger than those of the species on *E. glabratum* from Brazil, the sporangia are similar in size, and it is possible that these two fungi are identical.

SYNCHYTRIUM sp. on *Galactia mollis,* St. Martinsville, La., U.S.A.

(Leg. A. B. Langlois, 7–25–1895, Flora Ludoviciana No. *2405,* N. Y. Bot. Gard.)

Sori ovoid, 120–215 × 170–260 μ, or spherical, 130–220 μ. Sporangia several hundred per sorus, polyhedral, 11–21 μ in greatest diam. Zoospores unknown.

Compositely monogallic, sporangial galls abundant on both surfaces of leaves and on petioles, light-yellow, cupulate and aecidium-like after dehiscing, 130–180 μ high by 234–360 μ broad.

This species is labeled *S. decipiens* and resembles it fairly closely.

SYNCHYTRIUM sp. on *Galactia viridifolia,* Iguala, State of Guerro, Mexico. (Leg. Rose, Painter and Rose, 8–11–1905; spec. No. *9338,* N. Y. Bot. Gard.)

Sori ovoid, 200–280 × 320–400 μ, or spherical, 210–240 μ, light-yellow. Sporangia close to a thousand in the largest sori, polyhedral, 16–32 μ in greatest diam. with light-yellow content and delicate walls. Zoospores unknown.

Compositely monogallic, sporangial galls broadly mound-shaped, 143–358 μ high by 286–780 μ broad.

This species is labeled *S. decipiens* also, but its galls and sporangia are somewhat larger than those of the previous species and *S. decipiens.*

SYNCHYTRIUM sp. on *Vigna repens,* Dept. of Solala, Guatemala. (Leg. Kellerman, 15–2–1906; spec. in Arthur Herb., Purdue Univ.)

Sori ovoid to subspherical, 75–120 μ. Sporangia whitish to pale-yellow, polyhedral, 18–30 μ, exceptionally large ones 45 μ, with thin hyaline walls. Zoospores unknown.

Compositely monogallic, sporangial galls on both surfaces of leaves and on petioles, rarely confluent, orange to golden-yellow, almost circular in median sections, 90–215 μ, or oval to oblong, 110–133 × 125–200 μ.

SYNCHYTRIUM sp. on *Vigna vexillata,* Peralta, Costa Rico. (Leg. F. L. Stevens, 1927, spec. in herb. Univ. Ill.)

Sori usually ovoid, 200–312 × 320–400 μ. Sporangia several hundred per sorus, polyhedral, 18–33 μ in greatest diam. with thin hyaline walls and hyaline content. Zoospores unknown.

Compositely monogallic, sporangial galls usually scattered and separate on leaves, yellowish, mound-shaped, 208–260 μ high by 360–650 μ broad, sheath 3–6 cells thick.

Most of the sporangia of these two species on *Vigna* are larger than those of *S. decipiens* and *S. vignicola.* However, they, as well as Van der Byl's (**1928**) *S. dolichi* on *Vigna lutea* and Wiehe's (**1953**) *Syn-*

chytrium sp. on *Vigna unguiculata* in Africa, may relate possibly to *S. vignicola* (P. Henning) Gäumann which occurs on *Vigna sinensis* in East Africa.

SYNCHYTRIUM sp. on *Phaseolus atropurpurens,* Iguala, State of Guerro, Mexico. (Collector not indicated, 4–11–1903; spec. in Arthur Herb. Purdue Univ.)

Uromyces obscurans Holway, 1897. Bot. Gaz. **24**: 23–38.

Sori ovoid, subspherical or broadly pyriform, 265–364 μ diam. Sporangia several hundred per sorus, polyhedral, 8–23 μ in greatest diam., subspherical to oval, 12–16 × 18–20 μ, with thin hyaline walls and pale-yellow to lemon or whitish content. Zoospores unknown.

Compositely monogallic, sporangial galls abundant on both surfaces of leaves and on vines and pods, separate and scattered, or crowded and confluent and forming conspicuous protuberances, whitish to pale-yellow, cupulate and aecidium-like after dehiscing.

This species was determined as *S. decipiens* by Farlow in 1905, and on the herbarium envelopes he made a notation that the galls were described earlier by Holway (**1897**) as the aecidial stage of *Uromyces obscurans.* Holway described the aecidiospores (sporangia) as yellowish-red, globose or ellipsoidal, 17–20 × 17–25 μ and smooth. It was collected again by J. N. and J. S. Rose, 9–18–1906 (spec. No. *11465,* N. Y. Bot. Gard.) and A. S. Muller, 11–26–1950 (spec. No. *53033,* Arthur Herb. Purdue Univ.) on the same host at Vera Cruz, Mexico, and Honduras, respectively. As far as can be determined from the material at hand the fungi in these collections are identical. The author (**1954**) was unable to infect *P. atropurpurens* with *S. decipiens,* which indicates that Farlow's identification may be incorrect.

SYNCHYTRIUM sp. on *Phaseolus* sp., Jalapa, Mexico. (Leg. Holway, 10–2–1898; spec. No. *3166* Holway, Farlow Crypt. Herb., Harvard Univ.)

Sori spherical, 290–468 μ, or ovoid, 280–360 × 370–420 μ. Sporangia several hundred per sorus, polyhedral, 18–30 μ in greatest diam. with very delicate hyaline walls and faintly yellow content. Zoospores unknown.

Compositely monogallic, sporangial galls abundant on both surfaces of leaves and on petioles, separate or crowded and confluent, yellow, cupulate and aecidium-like after dehiscing, mound-shaped, 280–430 μ high by 386–620 μ broad; sheath 4–7 cells thick.

This species causes unusually large sori and galls with fairly thick sheaths.

SYNCHYTRIUM sp. on *Phaseolus* sp., Cabo Rojo, Puerto Rico. (Leg. F. L. Stevens, 6–13–1913; spec. in herb. Univ. Ill.)

Sori ovoid, 301–430 × 364–506 μ, pale-yellow. Sporangia several hundred per sorus, polyhedral, 18–31 μ, with thin hyaline walls and faintly yellow content. Zoospores unknown.

Compositely monogallic, sporangial galls usually separate and scattered or sometimes confluent, abundant on underside of leaf, mound-shaped and usually protruding conspicuously, 312–340 μ high by 360–600 μ broad; sheath 3–4 cells thick.

This, as well as the previous species on *Phaseolus*, may prove to be identical with *S. phaseoli* Weston.

SYNCHYTRIUM sp. on *Rhynchosia reticulata*, Cabo Rojo, Puerto Rico. (Leg. F. L. Stevens, 6–13–1913.).

Sori usually ovoid, 180–200 × 230–300 μ. Sporangia several hundred per sorus, polyhedral, 18–25 μ in greatest diam., exceptionally large ones 30–45 μ, with thin hyaline walls and yellow content. Zoospores unknown.

Compositely monogallic, sporangial galls usually separate and scattered, or sometimes confluent on both surfaces of leaves, brown, dome-shaped, 208–270 μ high by 260–416 μ broad, becoming cupulate after dehiscing; sheath 3–5 cells thick, inner sheath cells filled with resin-like substance.

This species resembles the one on *Eriosema glabratum* from Brazil and may be related to it. In this connection it may be noted that Ramakrishnan and Sundaram (**1954**) reported *S. atylosiae* on *Rhynchosia minima* in India, but their identification may prove to be incorrect in the light of Gäumann's (**1927**) tests, which indicate that *S. atylosiae* is limited in host range to species of *Atylosia*. Possibly, the species from Puerto Rico may be related to the one on *R. minima* in India. The sori and sporangia of both fungi are fairly similar in size.

SYNCHYTRIUM sp. on *Desmodium canescens*, Vicksburg and Brooklyn, Miss., U.S.A. (Leg. S. M. Tracy, 8–4–1888, and J. P. Kislanko, 6–20–1929, resp.; specs. in herb. N. Y. Bot. Gard.)

Sori subspherical to ovoid, 124–140 × 228–246 μ, and light-yellow. Sporangia several hundred per sorus, polyhedral, 16–30 μ, in greatest diam. with thin hyaline walls and faintly yellow content. Zoospores unknown.

Compositely monogallic, sporangial galls on leaves and petioles, separate and scattered or crowded and confluent, mound-, or dome-shaped when unopened, cupulate after dehiscing, oval to oblong and

circular in median cross section, 210–290 μ, on leaves, usually elongate and broadly pyriform, 240–290 × 380–400 μ, on petioles.

These two collections are labeled *S. fulgens* var. *decipiens* and *S. decipiens,* respectively.

SYNCHYTRIUM sp. on *Desmodium intortum,* Uyuca, Honduras. (Leg. A. S. Muller, 7–11–1950; spec. No. *53032,* Arthur Herb., Purdue Univ.)

Sori ovoid to subspherical, 180–265 μ diam. Sporangia up to several hundred per sorus, ovoid, oblong, 15–19 × 21–26 μ, subspherical, 16–23 μ, or polyhedral, 14–24 μ in greatest diam., with thin hyaline walls and almost hyaline content. Zoospores unknown.

Compositely monogallic, sporangial galls on leaves and petioles, usually scattered and separate, or crowded and confluent, dome-, or mound-shaped, becoming cupulate after dehiscence, circular to oval and oblong in median cross sections, 260–380 μ diam., usually elongate and broadly fusiform in sections of petioles, 265–332 × 467–600 μ diam.

This and the previous species may prove to be identical with *S. citrinum,* which has been reported on *Desmodium* sp., *D. axillaris,* and *D.* (*Meibomia*) *intorta* in Ecuador, Venezuela, India and Java. The dimensions of the sporangia given above correspond closely to those reported by Weston (**1930**) for *S. citrinum.* In Muller's material the contents of the sporangia are almost hyaline instead of lemon-yellow as reported for *S. citrinum,* but this lack of color may be due to fading from drying and storage. It may be noted here that Dr. C. Chardon, 8–27–1932 and 9–3–1932, collected similar fungi on *Meibomia intorta* at La Mesa and Merida, Puerto Rico, respectively, which Weston determined as *S. citrinum.* (Spec. in herb. Cornell Univ. and Farlow Crypt. Herb., Harvard Univ.)

It is apparent from the above descriptions that numerous members of *Woroninella* occur on leguminous hosts in the subtropics and tropics. Collectors have assumed that most of these are identical with *S. decipiens,* but this assumption is not justifiable in light of the limited host range of Peck's species. Some of the fungi may prove to be different species or possibly biological races of *S. decipiens.* Obviously, each species must be studied intensively from living material, and its host range must be determined before its identity and relationships can be established with more certainty.

SUMMARY

Synchytrium aecidioides (Pk.) Pat. and Lagerh. on *Psoralea mutisii* from Ecuador is a long-cycled species and differs markedly from Peck's

Uredo aecidioides or *S. decipiens* Farl. It is renamed *S. lagerheimii* sp. nov. and placed temporarily in the subgenus *Microsynchytrium*. In addition to *S. lagerheimii*, several other members of the subgenus *Woroninella* from other leguminous hosts are described, but they are not given specific names.

DEPARTMENT OF BIOLOGICAL SCIENCES,
PURDUE UNIVERSITY,
LAFAYETTE, INDIANA

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THE GENERA SACCOTHECIUM, PRINGSHEIMIA, PLEOSPHAERULINA AND PSEUDOPLEA [1]

L. E. WEHMEYER

(WITH 7 FIGURES)

The genera *Saccothecium, Pringsheimia, Pleosphaerulina, Pseudoplea* and the synonymy of their type species have been greatly confused in the literature. This confusion has revolved about a few species which have been variously interpreted. All of these species occur, often mixed, upon the same canes of *Rosa* or other woody shrubs and small trees. A recent upsurge in the interest in the pathogenic forms of this group on roses and various grasses and legumes makes it more urgent that this nomenclatorial confusion should be clarified. It is not the intent of this paper to discuss the details of synonymy, specific identity and hypothetical relationships of this group. This has already been done, from too many viewpoints, in the literature. Rather, it is to fix the identity of the generic types and critical species concerned. In order to clarify the discussion, these critical species will first be described, as determined by the writer. They are:

SACCOTHECIUM SEPINCOLA (Fr.) Fr. Summa Veg. Scand. 398. 1849. FIGS. 1–2.

Sphaeria sepincola Fr. Symb. Myc. **2**: 498. 1823; Obs. Myc. **1**: 181. 1815.

Sphaerulina sepincola (Fr.) Starb. Bot. Not. **1890**: 117. 1890; Bot. Centralbl. **46**: 261. 1891.

Pleosphaerulina sepincola (Fr.) Rehm, Ann. Myc. **10**: 539. 1912.

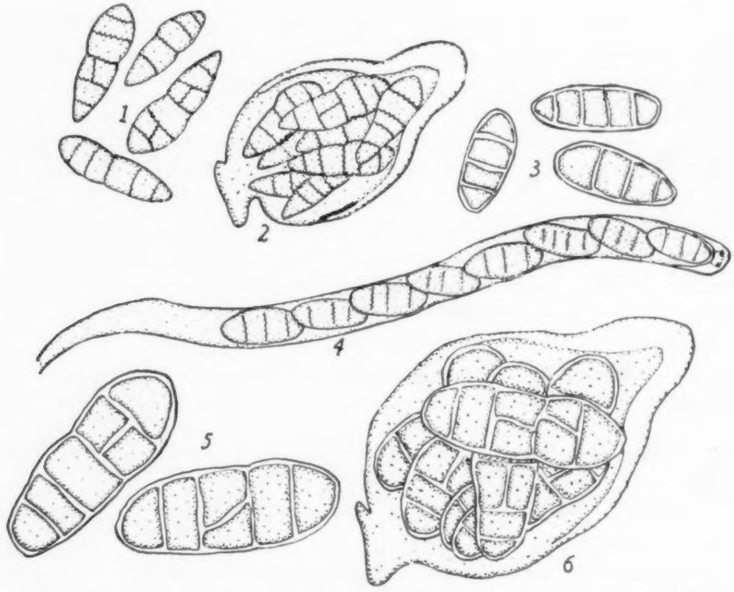
Pringsheimia sepincola (Fr.) Höhn. Ann. Myc. **18**: 96. 1920.

Ascostromata appearing on the surface as small hemispheric swellings of the periderm, these finally splitting open and appearing as angular to fusoid, black discs, 200–250 μ in diameter and usually seriately arranged, similar to a small *Botryosphaeria*, whitish within, spheric or flattened, variable in size, 150–250 μ, with an outer wall of coarse, dark-colored parenchyma 10–50 μ thick; no ostioles visible.

[1] Papers from the Department of Botany, University of Michigan No. 1060.

Asci saccate to broad-clavate becoming cylindric-clavate just before rupture, bitunicate, wall greatly thickened above, often swollen at the base into a claw-like attachment, 70–120 × 14–17 μ, borne in a fascicle upon a basal columella of large-celled parenchyma, with no intervening sterile elements.

Spores biseriate to triseriate, rarely ellipsoid, mostly fusoid to clavate-fusoid, asymmetric, broader above and narrow and more tapered below, straight or inaequilateral or curved, 3–5(–7)-septate, often asymmetrically so, with one or two septa above and two or three septa below



FIGS. 1–2. *Saccothecium (Sphaeria) sepincola* Fr. 1. Spores. 2. Ascus. FIGS. 3–4. *Sphaeria corticola* Fck. 3. Spores. 4. Ascus. FIGS. 5–6. *Pseudoplea gaeumannii* (Müll.) Wehm. 5. Spores. 6. Ascus.

the primary one, at which there is a central constriction, often with a vertical septum in one or two central cells, 13–23(–27) × (5–)6–7 μ.

This species belongs in the Ascoloculares and is characterized by the saccate, bitunicate, thick-walled asci and the crowded, clavate, asymmetric spores with 3–5 septa. It is commonly found on rose stems where the spores are 3–5-septate, but also occurs on the stems of other shrubs or small trees, in which case the spores may vary in size and septation.

SPHAERIA CORTICOLA Fck. Jahrb. Nass. Ver. Nat. **23–24**: 114. 1870. FIGS. 3–4.

Leptosphaeria corticola (Fck.) Sacc. Mich. **1**: 342. 1878.
Metasphaeria corticola (Fck.) Sacc. Syll. Fung. **2**: 166. 1883.
Pleosphaerulina corticola (Fck.) Rehm, Ann. Myc. **10**: 539. 1912.
Griphosphaeria corticola (Fck.) Höhn. Ann. Myc. **16**: 87. 1918.

Appearing on the surface as thickly scattered, often confluent, hemispheric pustules, blackening the surface tissues on some hosts. Perithecia sphaeriaceous, flattened-globose, 250–400 μ in diameter, lower wall usually thin (10–20 μ), pale brown, prosenchymatic, upper wall often thickened (50–100 μ), darker in color and partially parenchymatic; ostiole lined with periphyses.

Asci long-cylindric, apical wall slightly thickened and at first with a refractive ring in the apex, which turns blue with Melzer's reagent (KI), 95–125 × 7–9.5 μ, mixed with a few filiform paraphyses.

Spores uniseriate, oblong to ellipsoid, hyaline, mostly 3- (rarely 4- or even 5)-septate, straight or slightly inaequilateral, not constricted at the septa, symmetric, ends broadly rounded, rarely with a vertical septum in one cell, 13–20 × 6–7(–8) μ. The septa are faint in fresh spores and difficult to see, probably only divisions of the protoplast, but become distinct in Amman's or plasmolysed spores.

This species is one of the ascohymeniales, distinguished by the long-cylindric, unitunicate asci and the uniseriate spores with broadly rounded ends. It also occurs on *Rosa* but is found on other woody stems.

PSEUDOPLEA GAEUMANNII (Müller) Wehm. Myc. **47**: 164. 1955. FIGS. 5–6.

Pleospora gaeumannii Müller, Ber. Schweiz. Bot. Ges. **61**: 165. 1951.

Appearing usually on leaf spots or herbaceous stems as tiny, scarcely visible, brownish dots. Ascostromata small (50–100 μ), yellow-brown, thin-walled, membranous, with a punctate ostiole or a short beak, imbedded in the substrate.

Asci broadly saccate at first, elongating to clavate, swollen at the base and with a much thickened apical wall and a claw-like base, 50–100 × 25–35 μ.

Spores crowded, oblong to ellipsoid, mostly 4-septate, hyaline at first, finally pale brown, straight, with rounded ends, constricted at the septum, with a vertical wall in one or two cells, 35–45 × 11–16 μ.

This species is merely taken as an example of a group concerned here, which is often parasitic on leaves and has small membranous perithecia, large saccate bitunicate asci and crowded or triseriate spores which are large and with broadly rounded ends. A number of similar species have been described under various names. The spores vary in size and are from 3–5-septate, but the species are difficult to separate.

With these three names fixed as to their identity, we can proceed to summarize briefly their origin, usage and identity as referred to in the literature.

HISTORICAL

Fries (**1823**) described *Sphaeria saepincola* as follows: "Sphaeria saepincola, gregariis, peritheciis tectis globulosis opacis subrugosis, intus albis, ostiolo simplici pertusis.

Fries Obs. Myc. 1, p. 181. V.A.H. l.c., *S. corni* Sowerb. t. 370, f. 5.

. . . Ad ramos emortuos Rosae aliorumque fruticum (v.v.)."

Apparently none of the writers until Starbäck (**1891**) examined Fries's material of this *Sphaeria,* but merely guessed at its identity. Dr. Lennart Holm has kindly examined material of *S. sepincola* in the Fries Herbarium and reports (in litt.) that it is the *Sphaeria sepincola* of this paper, with the *Mycosphaerella* type ascostroma and asci. This is mentioned here to clarify the following discussion.

In Montagne (**1834**) we find the following:

"52. Sphaeria (Obtecta) Corni Montag., mss. non Sowerby (Pl. XIII, fig. 6)." A description is given and below it "Saccothecium Corni Fr. in litt. Novum genus." "Exs. Nob. n. 756." From Montagne's figures and a portion of the type collection, from Montagne, in the Berkeley Herbarium, at Kew, *Sphaeria corni* is a *Massaria, M. corni.* If this were to constitute a legitimate description of the genus *Saccothecium,* as some writers hold, *Massaria* would become a synonym of *Saccothecium.* However, in his discussion of *Sphaeria corni* Montagne says, referring to Fries' segregation of the genus *Sphaeria,* "Ne connaissant pas dans son ensemble de travail si vivement désiré, nous avon dû, pour ne pas sortis de notre plan, ajourner l'admission de ce genre dans lequel devront probablement rentrer quelques unes des espèces de la présent notice." Furthermore Fries (**1849**) described the genus *Saccothecium* as follows:

"XX Saccothecium Fr.

Perithecium integrum, obtectum, pore pertusam, includens saculos ovales pellucidos, paraphysibus fasciculatis cinctos ascique (secundarius) liberis sporifersque farctos.

1—S. saepincola Fr.
S. Corni Mont."

In a footnote he gives the genus as having profuse paraphyses, a character of *S. corni* but not of *S. sepincola,* and some four stages of development which are probably microscopic misconceptions of stages of maturity.

The *Sphaeria corni* Sow. to which Fries refers under *S. sepincola* (and which Montagne states is not his *S. corni*) is placed as *Didymella corni* (Sow.) Sacc. by Saccardo (**1883**), but Berkeley (**1852**) states that the type has curved spores which are not borne in asci.

There has been much discussion in the literature as to whether *S. corni* or *S. sepincola* should be taken as the type of the genus *Saccothecium.* From the foregoing it seems clear that Fries referred *S. corni* to his forthcoming genus *Saccothecium* in a letter to Montagne, and that Montagne did not mean his reference to *Saccothecium* Fr. to be a description of this genus, for he distinctly says that he does not know Fries' conception of the genus and therefore refrains from adding any other species to the genus. Fries in his description of *Saccothecium* puts his prior species *S. sepincola* first, as the type, and merely includes *S. corni* Mont. as a second species. There is obviously some confusion in the minds of both these authors as to what the limits of *Saccothecium* are, but inasmuch as the identity of the type of *S. sepincola* is now known and we are now following a type basis code, the writer believes that *S. sepincola* should be taken as the lectotype of *Saccothecium.*

Berkeley (**1852**) described *Sphaeria fuscella* (p. 325) and *Sphaeria intermixta* (p. 327) from the same twigs of *Rosa,* as follows:

"636. *S.* (*Obtectae*) *fuscella* n.s. Sparsa tecta; perithecia fuscis depressis; ascis linearibus obtusis; sporidiis uniserialibus oblongo-ellipticis quandoque curvulis triseptatis. On dead twigs of rose, Easton, Norths March 9, 1850."

"639. *S.* (*Subtectae*) *intermixta* n.s. Minutissima sparsa epidermide tantum tecta nigra; peritheciis depressis supra convexis perforatis; ascis clavatis; sporidiis biseriatis hyalinis clavato-fusiformibus triseptatis. On rose twigs mixed with *Sphaeria fuscella* but much smaller."

Under *S. fuscella,* Berkeley says that it differs from *S. sepincola* in its minute brown perithecia and even, elliptic, obtuse sporidia, and gives his notion of *S. sepincola* as what he formerly called *S. Gardineri.* He also states that *Sphaeria corni* Sow. has simple curved spores which are not in asci.

The interpretation of these species has caused a great deal of confusion in the literature. In the Berkeley Herbarium, at Kew, there

are two collections. The one from Easton, March 1850, is labelled both *S. fuscella* and *S. intermixta* and bears the two published figures of *S. fuscella* (fig. 20) and *S. intermixta* (fig. 24). It contains mostly perithecia of the fungus here called *S. corticola*. A few loose spores, not seen in asci, which resemble the fig. 24 of *S. intermixta* were seen in one mount. These spores were brown, 3-septate and 11–12.5 × 3.5–4.5 μ. On the second collection, labelled *S. fuscella,* there were found immature perithecia and asci of *S. sepincola* on one fragment and mature perithecia of *S. corticola* on another. It is difficult to interpret the foregoing evidence. *S. intermixta* seems to be the same as *S. sepincola* from the description, and it was so interpreted by Karsten and generally thereafter in the literature. The figure (24), however, resembles the small brown *Leptosphaeria*-like spores found in the type. *Sphaeria sepincola* occurs abundantly only on one piece of the second collection labelled *S. fuscella,* and there in an immature condition. The description and figures of *S. fuscella* appear to be that of *S. corticola* which is abundantly found on the type material, but Berkeley (in his discussion) gives the spores as "pale-brown" and as curved.

There are also seven collections in the Berkeley herbarium, including the *S. gardineri* mentioned, labelled *S. sepincola.* Most of these are immature, but they contain fungi with clavate asci and rather narrow fusoid, hyaline spores with 4 or more guttulae and 15–19 × 3.5–5 μ which may become septate at maturity. These agree with Berkeley's published account and figures on all seven packets but are misdeterminations of Fries' species.

It is obvious that Berkeley was confused as to this mixture of species and it seems best to consider them as nomina dubia and not use them as a basis for later species which have propagated this confusion.

Schulzer (**1866**) described a new genus *Pringsheimia,* with the type species *P. rosarum.* His description is brief "Sporen keulenformig, oben dick abwärts verdünnt, beiderseits abgerundet, septirt nicht gekerbt," but fits the clavate 3-septate spores figured, which fix it as the *S. sepincola* of Fries. No type material of this species has been seen.

Fuckel (**1870**, p. 114) described *Sphaeria corticola* as having "ascis stipitatis, cylindraceis, 96 Mik. long., 10 Mik. crass., sporidia 8, monosticha, oblonga, utrinque obtusa, triseptata, ad septa preparum constricta, 16 Mik. long., 6 Mik. crass., hyaline includentibus." His figures and a copy of his exsiccatus, Fung. Rhen. *911* (sub *Sphaeria vibratilis*) from the National Fungus Collection agree, and this is the fungus here described as *Sphaeria corticola* Fck. On this same page he says for *Sphaeria sepincola* "ascis cylindraceis stipitatis, 8 sporis; sporidiis

monostichis, oblongis, utrinque obtusis, triseptatis, hyalinis, 16 Mik. long., 5 Mik. crass.," which does not, of course, distinguish it from his *S. corticola.* Later (**1873**, p. 23) he says this was based on "unripe" material and corrects it as follows: "Ascis oblongis subsessilibus, 8 sporis 80–112 Mik. long., 14 Mik. crass. sporidia distichis, oblongo-clavatis, rectis, 2–4 tenuiter septatis. . . ."

Saccardo (**1883**) placed *Sphaeria sepincola* of Fries and Fuckel in *Metasphaeria sepincola* (Fr.?Fck.) Sacc., and gave Fuckel's (**1873**) revised description, and placed *Sphaeria corticola* Fck., in *Metasphaeria corticola* (Fck.) Sacc. In the same volume, *Sphaeria intermixta* B. & Br. and *Pringsheimia rosarum* Schulz. are placed as synonyms of his *Sphaerulina intermixta* (B. & Br.) Sacc.

Starbäck (**1891**) in discussing *Sphaeria sepincola* Fr. says that none of Fries' descriptions definitely fix this species and that the later descriptions of Fuckel, Saccardo and Winter were all based on the confused ideas of Fuckel. He examined the collection notated in Fries' handwriting, and states that it is the same as *Sphaerulina intermixta* (B. & Br.) Sacc. which he unites under the name *Sphaerulina sepincola* (Fr.) Starb. This failed to clear matters, however, for no one knew what *Sphaerulina intermixta* (B. & Br.) Sacc. was. This was cleared up by Dr. Holm's identification of Fries' fungus with the one here described as *Saccothecium sepincola.* The identity of *Sphaeria intermixta* B. & Br. still remains doubtful, but it is probably best left as a synonym of *S. sepincola.*

To compound this confusion, Saccardo (**1891**, p. 836), as a result of Starbäck's statement that *Sphaeria sepincola* Fr. is the same as *Sphaerulina intermixta* (B. & Br.) Sacc., published a combination *Metasphaeria sepincola* (B. & Br.) Sacc., which is meaningless, for Berkeley & Broome never used the epithet *sepincola* for one of their species, and the collections of *S. sepincola* in the Berkeley Herbarium ar misdeterminations of Fries' species and must receive a new name if used as the basis for a new species.

Passerini (**1891**), in this same year, described the genus *Pleosphaerulina* with *P. rosicola* as the type species. A collection marked *Pleosphaerulina rosae* Pass., in the Saccardo Herbarium, from the Passerini herbarium (on *Rosa canina,* prope Vigheffio) appears to be the type and is again the fungus here described as *Saccothecium sepincola.* At this point we can see that *Sphaeria sepincola, Sphaeria intermixta, Pringsheimia rosarum* and *Pleosphaerulina rosicola* should all be the same fungus and the genera *Saccothecium, Pringsheimia* and *Pleosphaerulina* are synonymous.

However, Saccardo (**1895**, p. 350) followed by Berlese (**1900**) placed a number of leaf-inhabiting parasitic fungi in the genus *Pleosphaerulina,* which differ in certain respects from *P. rosicola.* Berlese (**1900**) placed *S. intermixta* under *Pleosphaerulina,* but *S. sepincola* and *S. corticola* under *Metasphaeria* (his figures, Pl. 142, fig. 2, of *M. sepincola* are not *S. sepincola*). His figures of *Sphaerulina intermixta* (**1894**, p. 124), later *Pleosphaerulina* (**1900**, p. 98), are typical of *S. sepincola.* His figures (**1894**, Pl. 136, fig. 4) of *Metasphaeria corticola* are correct for *Sphaeria corticola.*

Rehm (**1912**, p. 538) was the first to recognize the true distinction between *Sphaeria corticola* Fck. and *Sphaeria sepincola* Fr. and the probable identity of *Sphaeria intermixta* B. & Br. with the latter, but be placed *S. corticola* as *Pleosphaerulina corticola,* which cannot be inasmuch as *S. corticola* is sphaeriaceous in structure. His statement that the spores of *P. corticola* are "später keulig . . . später 7-fach geteilt und im Alter mit einzelnen langsgeteilten Zellen" seems doubtful, although Jaap Fung. sel. exs. *424*, which he cites, does occasionally show vertical walls in oblong spores which are sometimes in somewhat clavate asci. Specimens of *Sphaeria sepincola* issued on *Cornus, Crataegus, Evonymus* and other woody hosts also show a good deal of variation in the size and septation of the spores, sometimes with a lack of vertical walls, but always clavate and in distinctly bitunicate asci. This has probably added to the confusion between these two species.

Höhnel (**1918**, p. 87) erected a new genus *Griphosphaeria* for *Sphaeria corticola* Fck. because of the upright parallel filiform character of the lateral perithecial walls. He also mentions a thin disc in the apex of the ascus which stains blue with iodine, which is probably the refractive ring often seen in these asci. Later in the same paper, Höhnel (**1918**, p. 162) described the new genus *Pseudoplea* for *Pleosphaerulina briosiana,* pointing out that *Pleosphaerulina* (Syn.: *Pringsheimia*) is a twig-inhabiting stromatic form, whereas *P. briosiana* is a leaf parasite with small pseudosphaeriaceous perithecia with few asci and interthecial strips of tissue.

Next, Rehm (**1918**) states that Fuckel's (**1870**, **1873**) two opinions as to the identity of *Sphaeria sepincola* were due to the presence again of both *Griphosphaeria corticola* and *Pleosphaerulina* (*Pringsheimia*) *sepincola* mixed on the same stems of his exsiccatus (Fung. Rhen. *2026*), and that Saccardo's *Metasphaeria sepincola* (B. & Br.) is a *Massarina* which is issued in Rehm Asc. *2143* as *Metasphaeria sepincola* (B. & Br.) forma *crataegi.*

Höhnel (**1920**, p. 95), in a discussion of *Sphaerulina* and *Pleo-*

sphaerulina, goes over the history just covered. He points out that *Sphaeria sepincola* should be the type of *Saccothecium*, but says that because Fries gives the asci as saccate and the paraphyses as numerous, and this does not apply to *S. sepincola*, *S. corni* must become the type, and that since *S. corni* is a *Massaria*, *Saccothecium* becomes a synonym of *Massaria*. (*S. sepincola* does have saccate asci but not numerous paraphyses, and as a type specimen exists, the genus *Saccothecium* should be tied to this type.) As a result of the exclusion of *Saccothecium* and the supposed identity of *Pringsheimia rosarum*, he makes the new combination *Pringsheimia sepincola* (Fr.) Höhn.

Petrak (**1921**, p. 28) emends Höhnel's genus *Pseudoplea* and considers it related to *Pleospora*. In the same paper (p. 32) he discusses and describes at length *Griphosphaeria corticola* (Fck.) Höhn. and states that its ascospores bud in the ascus. This has never been seen by the writer. He states it is related to the type of *Curreyella* and the Hypocreaceae. On page 36 he takes up *Pringsheimia sepincola* (Fr.) Höhn. and gives a description and synonymy of *S. sepincola* under this binomial. He would put it in the Dothioraceae. Next, on page 39, he discusses *Metasphaeria sepincola* Auct., which he bases on *Sphaeria sepincola* B. & Br. non Fr. He admits *S. sepincola* Fr. has priority, but because no one seems to know what this fungus is he brazenly takes the name for the Berkeley fungus, which he says is well known (sic). He puts this fungus in *Sclerodothis sepincola* (B. & Br.) Petr.

Clements & Shear (**1931**, p. 271) give *Metasphaeria sepincola* (Fr.) Sacc. as the type of the genus *Metasphaeria*, the only probable reason being that it is the first species listed by Saccardo which is based on a Friesian species. The concept of *Metasphaeria*, however, is quite distinct from that of *Saccothecium*.

Kirschstein (**1938**) reverts to the genus name *Saccothecium* and calls the bitunicate fungus *Saccothecium sepincola* Fr., which in the writer's opinion is the correct designation.

CONCLUSIONS

1. *Sphaeria sepincola* Fr. is one of the Ascoloculares or Loculoascomycetes, with bitunicate asci and clavate, 3–6-septate, often muriform spores. It was placed first by Fries under his description of *Saccothecium* and should be considered as the lectotype of *Saccothecium*. If *Saccothecium corni* is chosen as the lectotype of *Saccothecium*, *Massaria* becomes a synonym of *Saccothecium* and *Pringsheimia* would be the next in line of priority and the species would be *Pringsheimia sepincola* (Fr.) Höhn.

2. The types of the genera *Pringsheimia* Schulz. and *Pleosphaerulina* Pass. are identical with *Sphaeria sepincola* and become synonyms of *Saccothecium*.

3. The identity of *Sphaeria intermixta* B. & Br. is somewhat in doubt, but if not considered as a nomen dubium it should probably be a synonym of *S. sepincola*, as indicated by Berkeley's description and its occurrence on one of his co-types. It has also been so considered by Starbäck and most later writers.

4. *Sphaeria fuscella* B. & Br. is the second of the mixture of Berkeley's doubtful species. It appears to be the same as the later *Sphaeria*

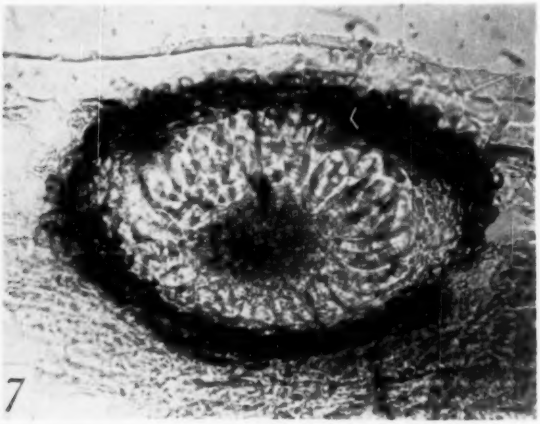


FIG. 7. Vertical section of a perithecium from the type of *Pleosphaerulina rosicola* Pass., showing the central columella bearing the fascicle of asci.

corticola of Fuckel, as indicated by Berkeley's figures and most of his description, and it occurs on both of his co-types. It is given as having curved pale brown spores, however, and has been placed in *Leptosphaeria* by later authors. It is best, perhaps, to conserve the name *corticola* for this fungus, as has been done throughout most of the literature. The true relationship of this species, beyond its being a member of the Sphaeriales or Euascomycetes, are yet to be determined. Höhnel's genus *Griphosphaeria* seems to be hardly of generic rank upon the basis of his distinction of wall structure.

5. *Sphaeria sepincola* B. & Br. and its later synonyms has no legitimate status. It is based upon one of Berkeley's vague entities, is a

misdetermination of Fries' species and should have a new name if distinct.

6. The genus *Pseudoplea* Höhn., based upon *P. briosiana*, is generically distinct from *Pleosphaerulina* (*Saccothecium*) and should be recognized. These distinctions are as follows: *Pleosphaerulina* occurs on woody stems and *Pseudoplea* upon leaves and herbaceous stems. *Pleosphaerulina* has ascostromata with thick dark colored walls; *Pseudoplea* has ascostromata with thin, membranous, pale brown walls. In *Pleosphaerulina* the asci are borne in a fascicle upon a basal columella of parenchymatic tissue and are ejected en masse. This was noted by Munk (**1953**) and well illustrated in his photograph (fig. 15) for his *Pringsheimia sepincola* (Fr.) Höhn. and illustrated (fig. 25) in a diagrammatic but rather exaggerated form by Müller and von Arx (**1950**). FIG. 7 is a photograph of a vertical section of a perithecium from the type of *Pleosphaerulina rosicola*, showing such a "columella." In *Pseudoplea* the asci are more or less crowded, parallel, separated by thin interthecial strips and ejected singly. In *Pleosphaerulina* the spores are clavate and tapered toward one end, whereas in *Pseudoplea* they are larger, ellipsoid to cylindric, with broadly rounded ends.

The genus *Pseudoplea* is related to or has been confused with such genera as *Wettsteinina*, *Sphaerulina*, *Metasphaeria*, and one group of *Pleospora*. The true distinctions and relationships of these genera must await a comparative study of at least the majority of the species concerned.

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STUDIES IN THE MYRIANGIALES. VII. ELSINOACEAE ON EVERGREEN EUONYMUS, ROSE AND ENGLISH IVY[1]

ANNA E. JENKINS[2] AND A. A. BITANCOURT

(WITH 2 FIGURES)

We are describing two new species of *Elsinoë* (Elsinoaceae), those on evergreen euonymus (*Euonymus japonicus*) and on rose (*Rosa*), besides revising our description of *Sphaceloma hederae*.[3]

Elsinoë euonymi-japonici sp. nov. FIG. 1

Maculae in foliis plerumque epiphyllae, rotundulae vel subirregulares, epiphyllae bene definitae, margine elevato aurantio-cinnamomeo, 0.2–2 mm in diam., per areas extensas coalescentes, majores umbone prominenti centrali rubro-brunneo vel obscuriori, e textura circumdanti interdum omnino a rima distinctae, zona inter rimam et marginem depressa albidaque; maculae hypophyllae elevatae, definitae, pulvinatae usque applanatae vel in majoribus centro depressae, rubro-brunneae, interdum cum decoloratione pallida vel albida; cancri in caulibus plerumque circulares usque elliptici, rugosi vel concentrice scindentes, griseo-albi, linea angusta aurantio-cinnamomea cincti, 0.5–3.5 mm in diam.; ascomata rotundata vel ovalia in ambitu, intraepidermicalia erumpentia, e textura pseudoparenchymatica hyalina composita, epithecio fusco tecta, ascos singulos vel plures continentia, $22–80 \times 12–15\,\mu$; asci globosi, $14–16\,\mu$ in diam., usque octospori; ascosporae hyalinae, 3-septatae, $11–13 \times 5–6\,\mu$.

Leaf spots mostly epiphyllous, roundish or slightly irregular, on the upper leaf surface well-delimited, with raised "orange cinnamon,"[4] waxy-appearing margin and, in the larger spots, a prominent center or knob set off by a rift or sometimes completely cleft, leaving a conical de-

[1] A contribution under the technical-assistance program operating in Brazil, under the joint administration of the Brazilian and United States Governments. For the United States the program is administered by the International Cooperation Administration.

[2] Dr. Jenkins, International Cooperation Administration, is assigned to the Technical Assistance Program in Brazil.

[3] We are indebted to those who collected or contributed the specimens cited, as well as to Miss Edith K. Cash for supplying the diagnoses in Latin and to Mr. J. A. Stevenson for his critical reading of the manuscript before publication.

[4] Names of colors in quotation marks are according to Ridgway (**10**).

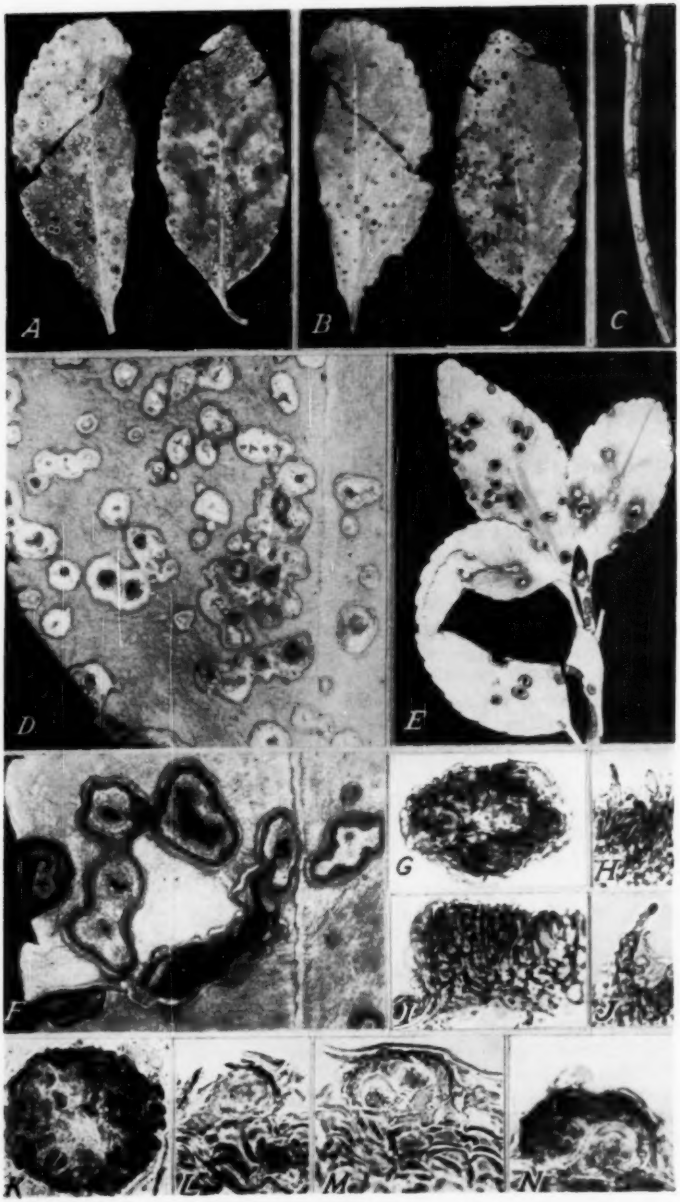

FIG. 1.

pression in its place, knob in some cases depressed at the center, in others fissured in one or more directions, "Van Dyke brown" or darker; zone between rift and periphery depressed, also whitened from the emptying of the epidermal cells, the spots individually 0.2–2 mm in diam., by coalescence more or less extensive, sometimes covering major part of the blade except for small irregular islets of healthy tissue; noticeable on the coalescent area, also present on individual spots, small rounded elevations resembling dots of white enamel; on the lower leaf surface, spots raised, well-delimited, pulvinate when small to flattened and depressed at the center when large, "walnut brown" especially at the periphery, with lighter, often whitish mottling due to the emptying of the epidermal cells.

Stem cankers often circular to elliptical, becoming wrinkled or fissured concentrically, grayish white, with slightly raised, "orange cinnamon" marginal line, 0.5–3.5 mm in diam., coalescent.

Ascomata round or oval as seen from top, intraepidermal, erumpent, or remaining covered by the outer wall of the epidermal cells, made up of hyaline pseudoparenchyma covered with a layer of larger cells with darkened outer wall forming an epithecium, containing one to several asci, 22–80 × 16–25 μ. Asci globose, 14–16 μ in diam., containing up to 8 hyaline, 3-septate ascospores, 11–13 × 5–6 μ.

On living leaves and stems of *Euonymus japonicus* L. (Celastraceae), United States of America and Japan.

Specimens examined: United States: Daytona Beach, Florida, June 8, 1954, C. R. Roberts, Comm. A. S. Muller (TYPE, IB *6432*, NFC *91267*);[5] Deland, Florida, December 27, 1954, C. R. Roberts, Comm. J. A. Stevenson (IB *6556*, NFC *91268*, specimen sterile or practically so). Japan, Matsudo, Chiba-ken, August 14, 1938, E. Kurosawa, Comm. K. Togashi (IB *5991*, NFC *91082*, only one ascoma seen, FIG. 1, G).

Obs. What we interpret as the *Sphaceloma* stage of *Elsinoë euonymi-japonici* is present on IB *5991* as well as on another specimen from

[5] Designations used in the citation of specimens: CU = Herb. Dept. Plant. Path., Cornell University. IB = Herb. Secção Fitopat., Inst. Biol. NFC = U. S. Nat. Fung. Coll. MSE = Jenkins and Bitancourt, Myriangiales selecti exsiccati.

FIG. 1, A–D. *Elsinoe euonymi-japonici* on *Euonymus japonicus* from Florida (Type, IB *6432*). A. Upper leaf surface. B. Reverse of A. C. On stem. D. Detail of A. E. Specimen from Japan (IB *5991*) on which a single ascoma was found. F. The leaf spot on IB *6417*, also from Japan. G. The ascoma from IB *5991*, viewed from above. H–J. Sphaceloma on the same specimen. K–N. Ascomata from IB *6432*, viewed from above (K) and in section (L–M). Magnification of A, B, C, E, × 1; D, F, × 5; G–N, × 500.

Japan, viz., Kurume City, Fukuoka, September 15, 1954, Coll. & Comm. S. Katsuki (IB *6417*) (FIG. 1, F, I–J). This *Sphaceloma* is treated separately by Kurosawa and Katsuki (**8**), who described it as *S. euonymi-japonici.*

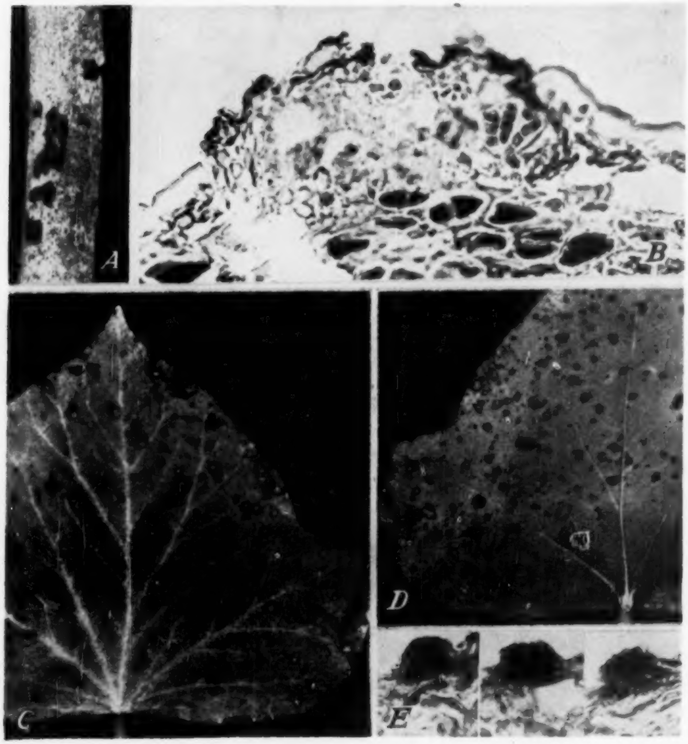

FIG. 2, A, B. *Elsinoe rosarum* on *Rosa ? pisocarpa* (IB *6842*). A. Infected stem, the several dark protuberances the original cankers, the numerous dark punctations, ascomata. B. Ascoma in section. C–E. *Sphaceloma hederae* on *Hedera helix* (IB *5903*). C, D. Parts of the upper (C) and lower (D) surfaces of the same leaf. E. Acervuli. Magnification of C, D, × 1; A, × 5; B, E, × 500.

Elsinoë rosarum sp. nov. FIG. 2, A, B

Ascomata in areis invasis emortuis ultra cancros parvos originales etiam ad cancros ipsos distributa, intraepidermicalia, erumpentia, in stratum exteriorem parenchymatis corticalis insidentia, e textura pseudoparenchymatica hyalina composita, epithecio tecta, 40–160 μ in diam., ca. 40 μ crassa; asci inordinate in pseudo-

parenchymate dispositi, globosi usque oblongi, 18–30 × 12–20 μ, usque octospori; ascosporae hyalinae, 1–3-septatae, 10–14 × 5–7 μ.

St. conid.

Sphaceloma rosarum (Pass.) Jenkins, Jour. Agric. Res. **45**: 330. 1932.

Phyllosticta rosarum Pass., Erbario Crittogamico Italiano, ser. 2, No. *1092*. Milan, 1881.

Ascomata abundant, scattered in the dry host tissue beyond the small stem cankers where the fungus has apparently spread and developed saprophytically, also on the cankers, intraepidermal, erumpent but remaining partly covered with the thick outer wall of the epidermal cells, resting on the outer layer of cortex-parenchyma cells, these filled with brown wound gum, made up of a hyaline pseudoparenchyma of isodiametric cells about 4 μ in diam. and a covering layer, epithecium, of thick-walled cells, 40–160 μ in diam., about 40 μ thick. Asci irregularly distributed in the pseudoparenchyma, globose or oblong, 18–30 × 12–20 μ, containing up to 8 ascospores. Ascospores hyaline, one to 3-septate, with upper cells broader and shorter than the lower cells, constricted at the middle septum, 10–14 × 5–7 μ.

On small living stems of *Rosa* ? *pisocarpa* A. Gray (Rosaceae), Det. W. H. Wheeler, Reedsville, Washington Co., Oregon, U. S. A., May 18, 1944, J. Roaf and C. G. Anderson *852*. Det. as ? *Elsinoë* sp. by W. H. W. (TYPE, IB *6482*, NFC *91264*).

Stem cankers of rose anthracnose, the disease caused by *Elsinoë rosarum*, have previously been recorded (**5**, **7**, **9**), though apparently not the spread of the pathogen beyond these small lesions. The leaf spot, also represented by IB *6482*, generally is more noticeable than the cankers. Among available illustrations are those in (**1**, **3**, **5**, **7**, **9**), where in one case (**9**, Fig. 1, A) the leaves are of *Rosa pisocarpa*.

SPHACELOMA HEDERAE Bitanc. & Jenkins, Jour. Wash. Acad. Sci. **36**: 420. 1946 (f. 2, G, H.) Char. emend. FIG. 2, C–E.

Leaf spots amphigenous, roundish or irregular, scattered or coalescing in groups of 4 or 5; on the upper leaf surface, raised, with "sorghum brown" margin and depressed center where the epidermis, desiccated and separated from the mesophyll, appears as a thin, greyish white, smooth or slightly wrinkled membrane, this becoming dotted with the dark sporodochia; pigmentation in leaf tissue around the spots producing a reddish halo-like effect in that area; on the lower leaf surface spots more prominent than on the opposite side, pulvinate when small, but depressed

at the center when larger, without well-defined margin, "fawn color," 0.2–4 mm diam.

Sporodochia minute, made up of a cluster of intimately coalescing, brown conidiophores, one- to several-celled, constricted at the septa, rarely branched, practically indistinguishable from the cells of the poorly developed underlying stroma, 18–80 × 14 μ. Conidia or terminal cells of the conidiophores oblong, with broad base and attenuated apex that sometimes is lighter colored than the rest of the cell, 8–11 × 5–6 μ.

On living leaves of *Hedera helix* L. (Araliaceae), United States (Maryland, Virginia, North Carolina and California) and Brazil (State of São Paulo).

Available specimens (other than those cited in the original description):

Maryland: Salisbury, March 12, 1951, R. A. Jehle (IB *5903*, NFC *91266*), also other specimens recorded elsewhere (**3**, f. 1, D; **4**).

Virginia: Kinloch, near Charlottesville, July 20, 1947, Freeman Weiss (IB *5134*, NFC *90451*, MSE *417*).[6]

North Carolina: Asheville, Grove Park Inn, August 28, 1947, G. H. Hepting and W. C. Snyder (TOPOTYPE, IB *5154*, NFC *90472*, MSE *416*).

California: Various specimens emanating from R. A. Jehle's discoveries of the fungus in the southern part of the state in 1946 (**2**) and 1947, for example, Altadena, December 11, 1946 (IB *5120*, NFC *90377*, MSE *330*), Los Angeles, Eagle Rock, November 30, 1946 (IB *5119*, NFC *90381*, MSE *331*) and others (1946–47) from the same place (MSE *413–415*).

Our two isolations of the *Sphaceloma* are from paratype material, that from Mar Vista, Calif. and from São Paulo, Brazil (**6**).

Our present description of *Sphaceloma hederae* is based particularly on the type specimen (Asheville, N. Car., Oct. 18, 1946, C. Westcott, IB *5101*, NFC *90270*) and the collection from Maryland cited above (f. 2, C–E). The emendation is required because in the original description the fructifications (**6**, I, *a*; J–M), for the most part at least, are of a secondary fungus, namely *Amerosporium trichellum* (Fr.) Lind.

In Argentina, Ringuelet (**11**) described intumescences on leaves of *Hedera helix* that in their general appearance and histology suggest the "scab" lesions induced by *Sphaceloma hederae*. They are smaller, how-

[6] MSE numbers *330* and *331* here cited are of Fascicle 7, issued by the U. S. Dept. Agr., Washington, D. C., 1947; likewise, numbers 413–417 are of Fascicle 9, U. S. Dept. Agr., 1949 [1953].

ever, and practically confined to the broad marginal area of the lower side of the leaf.

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NOMENCLATURAL NOTES. II. ON BULGARIA, PHAEOBULGARIA AND SARCOSOMA

Richard P. Korf [1]

The highly gelatinous Discomycetes originally placed in the genus *Bulgaria* have proved to be partly operculate (Pezizales) and partly inoperculate (Helotiales). Some authors have used the name for the inoperculate species, others for the operculate species.

Fries (**1822**) erected *Bulgaria* to include five accepted species (one operculate, four inoperculate) and a sixth, doubtful one. The name was an avowed substitute for the pre-starting-point *Burcardia* Schmidel [2] (**1797**), a later homonym. The lectotype of Fries' genus should doubtless be selected from the two species [3] recognized in Schmidel's treatment (International Code of Botanical Nomenclature, 1952, Appendix I, 2). Under the Code, the two eligible species may be cited *Bulgaria*

[1] Particular appreciation is due Dr. D. P. Rogers for certain references and for the loan of the type specimen of *Glococalyx bakeri*.

[2] Bibliographic Note: The author's name is often spelled Schmiedel, and the generic name is also cited as *Burkardia*, as the name appears on the plates. The date of publication of the genus is given as 1755 by Rehm (**1915**), as 1762 by Boedijn (**1932**), and as 1782 by von Höhnel (**1918**), but all appear to be erroneous. The first printing of this work is dated 1747; a second printing (apparently more common in the libraries) appeared with a new title page bearing the date 1762. Both of these printings contain only two volumes, consecutively paged 1–197 and include plates 1–50. The "second edition" consists of a third printing of the two volumes, both dated 1793, with new title pages and a rewritten leaf, pages [1–2], and a (new) third volume that is dated 1797. Volume 3 (pages 199–280 and plates 51–75) was published posthumously under the editorship of J. C. D. Schreber, and it is in this volume that *Burcardia* first appears. Rehm's date of 1755 stems from the statement (p. 262) that *B. globosa* was collected "Mensi Novembri 1755." Boedijn's 1762 date is that of the second printing of the first edition, in which the genus was not treated. Von Höhnel's 1782 date appears to be a *lapsus calami* for 1762.

[3] Of the remaining three species accepted by Fries, none appears to have been designated the lectotype of *Bulgaria*. *B. pellucens* (Schum. ex Pers.) Fr. appears to be lost, *B. pura* (Pers. ex Pers.) Fr. has been made the holotype of *Neobulgaria* Petr. (= *Ascotremella* Seaver), and *B. sarcoides* (Jacq. ex Gray) Fr. has been transferred to *Chlorosplenielia* O. Kuntze (apparently the valid name for the ascomycetous genus currently masquerading under the generic name *Coryne*—which is correctly a genus of Fungi Imperfecti).

globosa (Schmid. ex Pers.) Fr. (operculate) and *B. inquinans* (Pers. ex Hook.) Fr.[4] (inoperculate).

Recognition of the operculate nature of the asci in *B. globosa* led Rehm (**1891**) to publish the new genus *Sarcosoma,* credited to Caspary in a letter to Winter. *S. globosum* (Schmid. ex Pers.) Casp. *in* Rehm was the only species included, and hence is the holotype of the genus (*S. globosum* var. *platydiscus* Casp. *in* Rehm was also described). In this work *Bulgaria* was retained for the inoperculate species.

Seaver (**1928**), however, perhaps because it was the first species listed by Fries, decided to choose *B. globosa* as the [lecto]type of *Bulgaria,* thus making *Sarcosoma* an obligate synonym. He was apparently unaware that his choice of a lectotype was not the first, since he later (Seaver **1932**) objected to the Clements and Shear (**1931**) typification of *Bulgaria* with *B. inquinans* on the grounds that he had designated *B. globosa* as the type four years earlier. In fact, *B. inquinans* had been designated the type more than a century earlier by Brogniart (**1824**: 572) and again by Boudier (**1885**); further, the genus had already been emended to exclude *B. globosa* by Rehm (**1891**), Lindau (**1897**), Boudier (**1907**), and others. There can be no question but that the generic name *Bulgaria* must be used for the inoperculate species including *B. inquinans* and not for the operculate species as was done by such authors as Seaver (**1928, 1942**), Nannfeldt (**1932, 1949**), and Korf (**1949**).

In his treatment of *Bulgaria,* Seaver (**1928**) included two additional operculate species, *B. rufa* Schw. and *B. melastoma* (Sow. ex Gray) Seaver. In the opinion of Le Gal (**1951, 1953**), of Nannfeldt (*pers. comm.*), and of the author, *B. rufa* is not congeneric with *Sarcosoma globosum.* It is the type species of a new genus, *Galiella* Nannf. & Korf (Korf **1957**). *B. melastoma* is likewise not considered congeneric with *S. globosum* by these three authors, and is the lectotype of *Plectania* (Korf **1953, 1957**).

Believing that his designation of a lectotype for *Bulgaria* was valid, Seaver (**1932**) erected the genus *Phaeobulgaria* for the inoperculate species. He was followed in this by Nannfeldt (**1932**), who provided the combination *P. inquinans* (Pers. ex Hook.) Nannf. for the holotype of the genus. Since *Bulgaria* and *Phaeobulgaria* have the same type species, Seaver's genus is superfluous and an obligate synonym.

Three other generic names appear to require consideration from nomenclatural and taxonomic standpoints. (1) *Gloeocalyx* Massee,

[4] *Burcardia turbinata* Schmid. is a synonym, but the name chosen by Fries must stand (1952 Code, Art. 23, f).

based on *G. bakeri* Massee, was cited as a synonym of *Sarcosoma* by Boedijn (**1932**). Examination of the type specimen has convinced me that *G. bakeri* is a species of *Plectania,* and that *Gloeocalyx* is probably a synonym of *Plectania* sect. *Curvatisporae* (Korf **1957**). (2) Clements and Shear (**1931**) have cited *Voeltzkowiella* P. Henn. *in* Voeltz. (as "Voeltzknowiella," a *lapsus calami*) as a synonym of *Bulgaria* Fr. The type specimen of Hennings' species is apparently lost (*cf.* also Le Gal, **1953**: 11), and no additional collections seem to have been recorded. The type of the generic name and of the holotype species (*V. madagascariensis* P. Henn. *in* Voeltz.) is now the original description and plate (Hennings **1908**). My examination of these indicates a fungus quite close to, if not identical with, *Bulgaria inquinans.* The brown, rather small ascospores, the paraphyses, and the presumably inoperculate asci all point to such a relationship. The apothecium appears to arise, however, from a membranaceous-gelatinous hyphal mat on which "conidia" are also produced. Until specimens of this fungus are rediscovered, it seems best to regard *Voeltzkowiella* as a probable synonym of *Bulgaria.* (3) When Rehm (**1915**) and von Höhnel (**1918**) revived the generic name *Burcardia* Schmid. they were using what is today a pre-starting-point name. *B. globosa* was recognized as the holotype of the genus, thus making *Burcardia* sensu Rehm an obligate synonym of *Sarcosoma.*

In two recent treatments by Le Gal (**1951, 1953**), the generic name *Sarcosoma* has been taken up in a different sense. She has used the name for a number of species that have been placed in *Sarcosoma,* but which she recognized are not congeneric with *S. globosum.* Following Seaver's (**1928, 1932**) treatment of the nomenclature and noting Nannfeldt's (**1949**) acceptance of Seaver's typification, she reasoned that with *S. globosum* removed to *Bulgaria,* the generic name *Sarcosoma* could still be used. She misused the neotype concept by excluding the holotype, in opposition to the Code, and by recognizing *S. javanicum* Rehm *in* Hennings as the "lectotype" of *Sarcosoma* (*i.e.,* as the *neotype,* since that species was not described until two years after the genus was erected).[5]

Under the International Code, the following must be the acceptable synonymy and typification:

[5] At least in part, Mme. Le Gal was led astray by uncertainty as to the valid date of publication of *Sarcosoma* in the Rehm volume of the Kryptogamen-Flora. The completed volume bears the date 1896 on the title page, but it was issued in separate fascicles which appeared between 1887 and 1896. If *S. javanicum* had been published before *S. globosum,* as she apparently believed, her typification would have been correct.

BULGARIA Fries (**1822**). (Helotiales)

[≡*Burcardia* Schmidel (**1797**), *pre-starting point;* non *Burcardia* Schreber 1789, nec *Burcarda* Gmelin 1791.]

≡*Peziza* Hook. [subgen.]†††††† *Burcardia* (Schmid.) ex Persoon (**1822**).

[≡*Burcardia* (Schmid. ex Pers.) Fries (**1822**) *pro synon.*]

≡*Burcardia* (Schmid. ex Pers.) Rehm (**1915**) *excl. species.*

≡*Phaeobulgaria* Seaver (**1932**).

= ? *Voeltzkowiella* P. Hennings (**1908**) *in* Voeltz.

SPECIES LECTOTYPICA: *B. inquinans* (Pers. ex Hook.) Fr., designated by Brogniart (**1824**).

SARCOSOMA Caspary *in* Rehm (**1891**). (Pezizales)

[≡*Burcardia* sensu Rehm (**1915**), non sensu stricto.]

[≡*Bulgaria* sensu Seaver (**1928, 1942**), *pro parte typica,* non sensu stricto.]

SPECIES HOLOTYPICA: *S. globosum* (Schmid. ex Pers.) Casp. *in* Rehm.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

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TWO BULGARIOID GENERA: GALIELLA AND PLECTANIA

RICHARD P. KORF [1]

A NEW GENUS, GALIELLA

As I pointed out in a previous communication (Korf **1957**), Mme. Le Gal has utilized the generic name *Sarcosoma* in a sense which excludes its holotype. Since *Sarcosoma* is unavailable for the fungi treated by her (Le Gal **1951, 1953**), and no other generic name seems to be valid, a new genus is now proposed to accommodate these species.

A major contribution to this group of Discomycetes in recent times has undoubtedly been the meticulous work of Mme. Le Gal, and Professor Nannfeldt joins me in proposing the generic name in her honor. *Bulgaria rufa* Schw., a common North American species, is designated the type; it is a species Mme. Le Gal has worked with, and is more familiar to the authors than are *S. javanicum* and its typically tropical allies. The genus has essentially the circumscription of *Sarcosoma* sensu Le Gal (**1953**), but *S. rhytidia* (Berk. *in* Hook.) Le Gal and probably *S. turbinatum* Wakef. are excluded (see below, under *Plectania*). The genus embraces the bulgarioid species with finely to grossly warted spores, the markings being callose-pectic in composition and reacting to cotton blue dyes (Korf **1952**).

Galiella Nannfeldt & Korf, gen. nov. (Sarcoscyphaceae)

Apothecia epixyla, plerumque magna, sessilia vel breviter stipitata, carne egregie crassa gelatinosaque, plerumque hemisphaerica vel crateriformia; sicca maxime contracta, facie plicata vel rugosa. *In sectione* hypothecium plerumque distinctum; excipulum medullare e textura intricata in matrice gelatinosa sita, saepe crassissimum, aliquando hypharum parallelarum zonis praeditum; excipulum exterum e textura intricata vel ad texturam angularem vel globulosam vergens, cellulis brunneotunicatis, capillas gignens. *Capillae* brunneo-tunicatae, longae vel breves, leves vel asperae, flexuosae, specie viventes. *Hymenium* color pallido ad rufum vel griseoatrum vergente, aliquando, praecipue siccum, fere atrum. *Asci* magni, longi, deor-

[1] My thanks are due Dr. D. P. Rogers, New York Botanical Garden, for preparation of the Latin diagnoses. I am deeply indebted to Prof. J. A. Nannfeldt, Uppsala University, for his continued help and encouragement. Some of the concepts expressed here have been independent conclusions; but some were undoubtedly first suggested to me by conversation and correspondence with him.

sum attenuati, modice crasse tunicati, suboperculati, liquore iodi haud cyanescentes. *Ascosporae* uniseriatae, unicellulares, magnae, punctis e materie callosa-pectica tenuibus vel crassis ornatae. *Paraphyses* graciles, interdum ramosae vel anastomosantes.

SPECIES HOLOTYPICA: **Galiella rufa** (Schw.) Nannf. & Korf, comb. nov., basionym: *Bulgaria rufa* Schw., Trans. Amer. Phil. Soc. II **4**: 178. [1832.]

SPECIES ALIAE: **Galiella javanica** (Rehm *in* P. Henn.) Nannf. & Korf, comb. nov., basionym: *Sarcosoma javanicum* Rehm *in* P. Henn., Hedwigia **32**: 226. 1893.

Galiella thwaitesii (Berk. & Br.) Nannf. comb. nov., basionym: *Rhizina thwaitesii* Berk. & Br., Jour. Linn. Soc. [Lond.], Bot. **14**: 102. 1875.

Galiella celebica (P. Henn. *in* Warb.) Nannf. comb. nov., basionym: *Bulgaria celebica* P. Henn. *in* Warburg, Monsunia **1**: 30. 1900.

Several additional species surely belong in the genus, but type studies are required to untangle the nomenclature.

Galiella may be clearly distinguished from the two other, fairly common genera of operculate Discomycetes with a gelatinous consistency, *Sarcosoma* and *Plectania,* in possessing ascospores with callose-pectic markings. It also differs from *Sarcosoma* in having smaller apothecia, and in not possessing a gelatinous fluid within the apothecium. It may be further differentiated from *Plectania* in having usually larger, lighter colored apothecia, and in having a much more extensive, gelatinous medullary excipulum.

ON PLECTANIA FCKL. EMEND. SACC.

As I have shown earlier (Korf **1953**), *Plectania* must be typified by *P. melastoma,* a black, bulgarioid species included in *Bulgaria* by Seaver (**1928, 1942**). (*Plectania* sensu Seaver is in part *Sarcoscypha* and in part *Microstoma.*)

Both Kanouse (**1948**) and Nannfeldt (**1949**) have used the generic name *Plectania* Fckl. emend. Seaver for the scarlet-cup, *Sarcoscypha coccinea,* and its allies. For *Plectania* Fckl. emend. Sacc. they have applied the more recent name, *Rhizopodella* (Cooke) Boud. This genus is also typified by *P. melastoma,* however, and is thus an obligate synonym of *Plectania* (Korf **1953**).

Examination of the type specimen of *Glococalyx bakeri* Mass., the holotype of the genus *Glococalyx,* on deposit in the New York Botanical Garden has shown this also to be allied to *P. melastoma.* It differs particularly in its suballantoid ascospores, and as Prof. Nannfeldt has

pointed out (*pers. comm.*), is probably not distinct from *Peziza campylospora* Berk. *in* Hook. In my opinion, *Gloeocalyx* is a synonym of *Plectania*.

In the supplemented edition of his book, Seaver (**1942**: 319, 320) synonymized *Sarcosoma cyttarioides* Rehm *in* Durand with "*Bulgaria*" *melastoma*. Ample material of Rehm's species (to be issued in Korf, Discomyceteae Exsiccatae) has shown that it is indeed congeneric with "*B.*" *melastoma* (*i.e.*, it is a *Plectania*), but that it has transversely furrowed spores and is thus quite distinct.

Although neither Prof. Nannfeldt nor I have completed our independent studies of this genus, it seems advisable to present a fairly complete synonymy of the genus at this time. Certain new combinations are proposed, two of which are provided for North American species; a division of the genus into three sections, based primarily on ascospore characters, seems warranted, as outlined in the following key:

I. Ascospores smooth, without transverse furrows.
   A. Ascospores ellipsoid....................................*Sect.* **Plectania**
   B. Ascospores suballantoid............................*Sect.* **Curvatisporae**
II. Ascospores with transverse (or longitudinal ?) furrows, ellipsoid.............
   ....*Sect.* **Plicosporae**

PLECTANIA Fuckel, Jahrb. Nass. Ver. Naturk. **23–24**: 323. [1870.] *emend.* Saccardo, Syll. Fung. **8**: 163. 1889 (*non emend.* Seaver, N. Am. Cup-fungi (Op.) 190. 1928, ≡ *Sarcoscypha* (Fr.) Boud.).
≡ *Peziza* ser. *Lachnea* subgen. *Rhizopodella* Cooke, Mycogr. **1**: 260. 1879.
≡ *Peziza* subgen. *Plectania* (Fckl.) Sacc., Bot. Centralbl. **18**: 215. 1884.
≡ *Rhizopodella* (Cooke) Boud., Bull. Soc. Myc. Fr. **1**: 103. 1885.
= *Gloeocalyx* Mass., Kew Bull. Misc. Inf. **1901**: 155. 1901.

SPECIES LECTOTYPICA: *Calycina melastoma* (Sow.) ex Gray (≡ *Plectania melastoma* (Sow. ex Gray) Fckl.) effectively selected by Saccardo, Syll. Fung. **8**: 163. 1889, when he divided the genus.

Section **Plectania** Korf, sect. nov.

Genotypi, *P. melastomatis*, characteribus praedita; sporae ellipsoideae, leves.

SPECIES HOLOTYPICA: *P. melastoma* (Sow. ex Gray) Fckl.

SPECIES ALIAE: **Plectania nannfeldtii** Korf, nom. nov., basionym: *Paxina nigrella* Seaver, N. Am. Cup-fungi (Oper.) 208. 1928, non *Plectania nigrella* (Pers. ex Pers.) Karst., named in honor of Prof.

J. A. Nannfeldt, who first pointed out to me the relationships of this species; also the species of "*Bulgaria*" described by Seaver and Waterston (**1946**: 182) from Bermuda, which appears to be undescribed; also certain other species currently being worked out by Prof. Nannfeldt.

Section **Curvatisporae** Korf, sect. nov.

Sicut generis sectio typica, sed sporae suballantoideae.

SPECIES HOLOTYPICA: **Plectania campylospora** (Berk. *in* Hook.) Nannf., comb. nov., basionym: *Peziza campylospora* Berk. *in* Hook., Bot. Antarctic Voy., Part II, Fl. Novae-Zeland. **2**: 200. 1855. A probable synonym of this section is *Gloeocalyx* Mass. There is perhaps only one valid species with curved spores.

Section **Plicosporae** Korf, sect. nov.

Sicut generis sectio typica, sed sporarum tunica sulcis transverse (vel in longitudinem ?) incisis ornata.

SPECIES HOLOTYPICA: **Plectania cyttarioides** (Rehm *in* Durand) Korf, comb. nov., basionym: *Sarcosoma cyttarioides* Rehm *in* Durand, Jour. Myc. **9**: 104. 1903.

SPECIES ALIAE: **Plectania rhytidia** (Berk. *in* Hook.) Nannf. & Korf, comb. nov., basionym: *Peziza rhytidia* Berk. *in* Hook., Bot. Antarctic Voy., Part II, Fl. Novae-Zeland. **2**: 200. 1855; also several other species with transversely furrowed spores that are being worked out by Prof. Nannfeldt, including *Urnula platensis* Speg. which surely belongs here. Transfer of these species awaits his further studies. *Sarcosoma turbinatum* Wakef. with longitudinally furrowed spores may possibly belong in this section.

DEPARTMENT OF PLANT PATHOLOGY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

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A NEW SPECIES OF XYLARIA [1]

JULIAN H. MILLER AND L. W. NIELSEN

(WITH 2 FIGURES)

It is rather unusual to find a species of *Xylaria* growing on grass roots. This one developed in a blue grass (*Poa pratensis* L.) lawn in a city lot in Raleigh, N. C., during the summer of 1956. There was an area of dying grass, 40 to 60 feet in diameter, in the center of the lawn, no part of which was shaded by trees. It was thought the death of the grass was due to a burrowing web worm and the presence of the *Xylaria* in the area was probably coincidental.

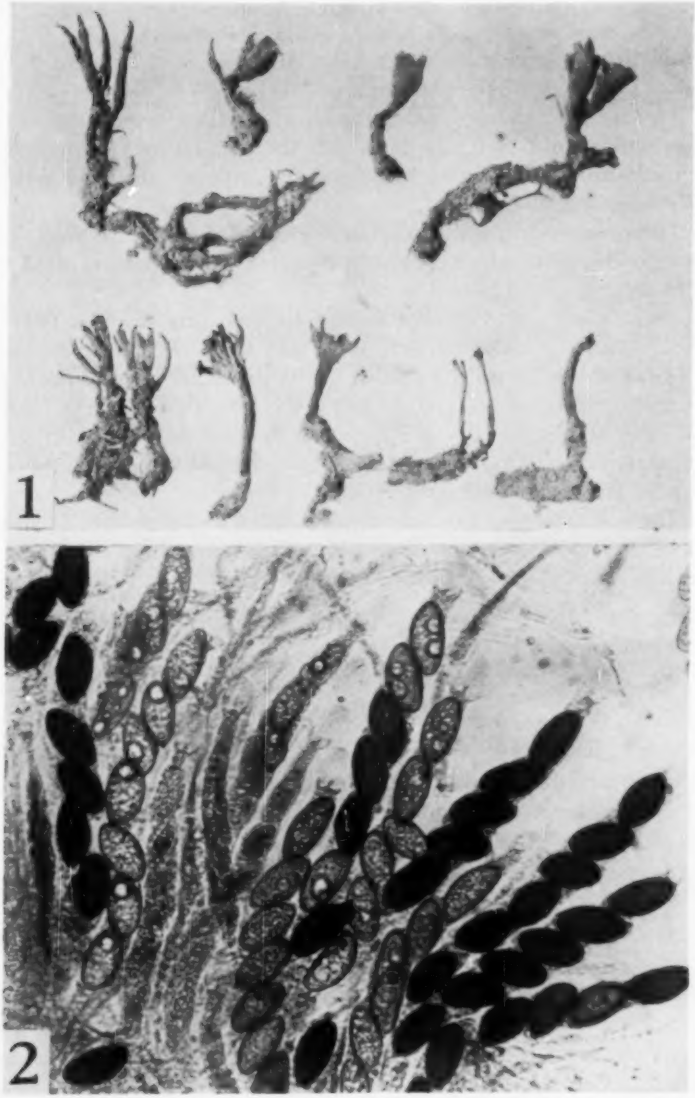
The only organic materials present in addition to the dead grass roots and clippings were a few oak and pecan leaves. No stable manure had been applied. The habitat is uncommon, as most named species have been found on wood. There have been a few recorded on other substrates such as decayed fruits, nuts, leaves and even termite nests. The only other ones described on grass roots are *X. graminicola* Ger. from New York and *X. rhizophila* Cke. and Mass. from Australia.

Xylaria multipartita sp. nov.

Stroma erectum, simplex vel ramosum et furcatum, teres vel compresso-dilatatum, fusco-nigricans, glabrum, basi sclerotiiformi, 30–50 × 1.5–6 mm; clavula perithecigera apice obtuso-crenato vel attenuato-acuto; peritheciis immersis, globosis, 300–400 μ latis, ostiolis atris, rotundis prominentibus; ascis cylindricis, p. sp. 66–120 × 10–12 μ, breviter stipitatis, 4–8 sporis; ascosporidiis oblique monostichis, oblongo-fusoideis, inaequilateralibus, atrofuscis, 15–18 × 8–10 μ; paraphysibus simplicibus, filiformibus, 2–5 μ latis.

Stroma dark brown, white inside, usually solitary and branched with apices divided, terete, attenuate or compressed-dilated with crenate margin, smooth, with stipe connected with a subterranean sclerotium, 30–50 mm high and 1.5–6 mm wide, with the sclerotium 30–50 × 1.5–3 mm. Perithecia coarsely papillate, peripheral, developing from the apex downward to almost the base of the stipe, conic-globose, 300–400 μ in diameter. Asci 4–8-spored, cylindric, p. sp. 60–120 × 10–12 μ, with short stalk, 20–30 μ long. Ascospores obliquely uniseriate, inaequilaterally

[1] Journal series paper no. 27, College Agricultural Experiment Station, University of Georgia, Athens, Georgia.



FIGS. 1–2. *Xylaria multipartita.* 1. Stromata with some attached to sclerotia, nat. size. 2. Group of asci and ascospores, × 666.

elliptical with obtuse ends, dark brown to opaque, 15–18 × 8–10 μ. Paraphyses broad bands, 2–5 μ wide, quickly deliquescing.

Habitat on dead grass roots (*Poa pratensis* L.), Raleigh, N. C. Coll. L. W. Nielsen, July 21, 1956.

This species developed rather thickly all over the dying spots in the grass during June and July, but when the temperatures became very high and rainfall low in early August the stromata shriveled and all but disappeared.

There was evidently some nuclear degeneration within the ascus. In some perithecia the asci were chiefly 4-spored, but in most of them the spore number varied from 4 to 8.

Other species of somewhat similar stromal form, such as ones in the *X. multiplex* (Kze. ex Fr.) Berk. and Curt., *X. arbuscula* Sacc., *X. apiculata* Cke. series, are distinct due to the rather broad ectostromal bands diagonally placed, and *X. hypoxylon* (L. ex Fr.) Grev. differs in smaller spores (9–12 × 4–5 μ). Also none of these normally grow on grass roots. *X. graminicola* and *X. rhizophila* have spores only 10 × 5 μ and 8–10 × 2–3 μ respectively.

The type collection has been divided and deposited in the Mycological Herbarium, University of Georgia, the Mycological Herbarium of the New York Botanical Garden and in the Mycological Herbarium of the U. S. Department of Agriculture.

THE UNIVERSITY OF GEORGIA,
ATHENS, GEORGIA
AND
NORTH CAROLINA STATE COLLEGE,
RALEIGH, NORTH CAROLINA

AN UNDESCRIBED SPECIES OF PYRENOCHAETA ON SOYBEAN

Robert B. Stewart

(with 1 figure)

Soybeans, *Glycine max* (L.) Merr., are not commonly grown in Ethiopia but have been cultivated experimentally since 1953 by the Jimma Agricultural Experiment Station, Jimma, Ethiopia. During this period the experimental plots have shown an increasing percentage of leaf spotting, due to what is apparently an undescribed species of *Pyrenochaeta,* which is described below. This is the most prevalent disease of soybeans in this locality.

Pyrenochaeta glycines sp. nov.[1]

Maculae foliicolae, subcirculares, canae aut obscure brunneae, cum margine obscuriore, aliquando cum corona chlorotica; centrum pycnidiis parvis nigris sparsum nudis oculis visibilibus. Pycnidia amphigena, erumpentia, sphaerica aut plana, cellulis flavo-brunneis composita ad ostiola obscurioribus, 110–160 μ in diametro; ostiolis irregularibus, circiter 15–20 μ in diametro; setae paucae, rigidae, erectae, circum ostiola collectae, in apicem attenuatae, 0–1-septatae, acumine rotundo vel obtuso, $4\text{–}5 \times 30\text{–}110\ \mu$ (6–7 μ crass. ad basim); sporulae hyalinae, ovales vel breviter cylindratae, rectae vel curvatae, $2\text{–}3 \times 4.5\text{–}7.5\ \mu$.

Collections: On *G. max* September 15, 1955. Jimma, Ethiopia. Robert B. Stewart. Type: Bureau of Plant Industry, Mycological Collections, Beltsville, Maryland, U. S. A. Co-types: Herbarium of the Royal Botanic Gardens, Kew, England. On *G. javanica* L. October 10, 1955. Jimma, Ethiopia. Robert B. Stewart. Bureau of Plant Industry, Mycological Collections.

The leaf spots occur from seedling stage to maturity, and first appear as small, dark reddish-brown, circular to irregular lesions. The older lesions are necrotic, roughly circular (occasionally somewhat irregular), gray to tan or dark brown in the center with a dark brown to almost black, narrow border. The lesions are sometimes surrounded by a chlorotic halo. The center of the lesion is sprinkled with small black pycnidia plainly visible to the unaided eye. In old lesions this central

[1] Latin diagnosis prepared by Dr. D. P. Rogers, New York Botanical Garden.

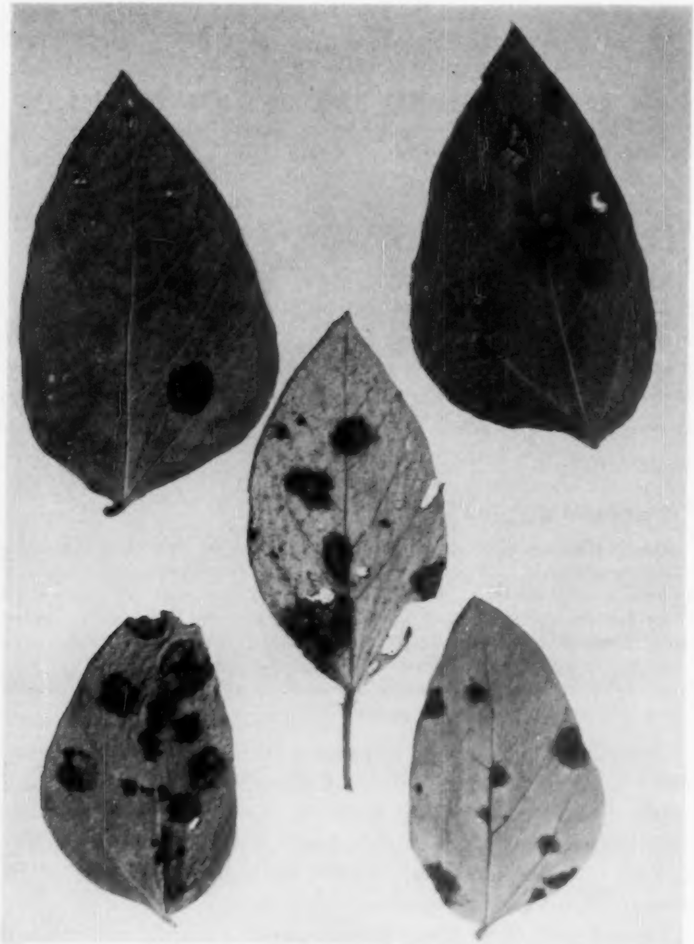


FIG. 1. Leaves of soybean variety S-100 showing typical necrotic lesions caused by *Pyrenochaeta glycines.*

necrotic area may break up and fall out, causing the leaf to have a ragged appearance. The lesions measure up to 2 cm in diameter but may coalesce to cover the greater portion of the leaf surface. Affected leaves become chlorotic and abscise.

Leaf abscision is the most damaging aspect of the disease and has

caused up to 75 percent defoliation of some varieties of soybean. The lesions are distinctive but somewhat similar to those caused by *Alternaria* sp. and arsenical injury. Varieties with pale green leaves are, in general, more susceptible to the disease than those with darker leaves. The spots are fewer and remain smaller on the darker leaves.

The pycnidia are erumpent, composed of yellow-brown cells which are darker about the ostiole, spherical to flattened, and measure 110–160 μ in diameter. A few stiff, erect, dark brown seta are clustered about the ostiole. The seta are widest near the base and taper toward their apices, which are rounded or obtuse. They are aseptate to 1–2-septate and measure $4\text{–}5 \times 30\text{–}110\ \mu$ ($6\text{–}7\ \mu$ wide at the base). The pycnospores are hyaline (greenish-yellow in mass), oval to short cylindrical, straight to slightly curved, and measure $2\text{–}3 \times 4.5\text{–}7.5\ \mu$.

The same organism has been seen on an uncultivated host, *Glycine javanica* L. in this locality. The symptoms on this host are the same as those on soybean except that the leaves tend not to abscise, but to remain on the host and give the plant a very ragged appearance. It is likely that the organism spread to soybean from this indigenous host.

TEXAS AGRICULTURAL EXPERIMENT STATION,
COLLEGE STATION, TEXAS

THE GENUS SEBACINA [1]

Marion D. Ervin [2]

The genus *Sebacina* was established by L. R. and C. Tulasne (Jour. Linn. Soc. Bot. **13**: 36. 1871) to accommodate two species, *Corticium incrustans* Pers. and *C. caesium* Pers. The next year the Tulasne's paper was printed in French (Ann. Sci. Nat. Bot. V. **15**: 215–235. 1872) and the illustrations added. The first species, *Sebacina incrustans* (Pers. ex Fries) Tulasne, is a well-known and widely distributed species exhibiting great variation, which has led students to apply additional names to it (see Martin, Univ. Iowa Stud. Nat. Hist. **19**(3): 53. 1952). Clements and Shear (Gen. Fungi 342. 1931) cite *Sebacina laciniata* [Bull.] Bres. as the type of the genus. It is true that the Tulasnes cited *Clavaria laciniata* Pers. as a synonym, but there can be no doubt that the species the authors had in mind in describing the new genus was what they had previously referred to *Corticium incrustans*. *Corticium caesium* is less fully described, appears to be much less common and its identity is by no means so firmly established. Bourdot and Galzin (Hymén. Fr. 41. 1928) cite *Sebacina caesia* (Pers.) Tul. as a subspecies, following *S. laciniata* (*i.e., S. incrustans*); the spore size as given and the notation that clamp-collections are lacking tend to support this disposition. The Tulasnes had never, of course, heard of the combination *S. laciniata*. It seems clear that the type of *Sebacina* must be *Corticium incrustans* Pers., as indicated by Burt (Ann. Missouri Bot. Gard. **2**: 749. 1915). The genus has been widely accepted, but the generic limitations have varied very greatly.

Although certain phases of *Sebacina incrustans* bear free lobes, and thus approach in this respect, as in the nature of the hyphae and basidia, the species of *Tremellodendron*, the species is usually resupinate, and the tendency has been to include in *Sebacina* all resupinate fungi with cruciate-septate basidia and with a waxy or gelatinous texture. Patouillard (Bull. Soc. Myc. Fr. **8**: 120. 1892) established the genus *Heterochaete* for *Sebacina*-like fungi with spine-like clusters of sterile hyphae

[1] Based on a thesis submitted to the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Dr. Marion E. Williams, Fisk University, Nashville 5, Tennessee.

emerging from the hymenium. Bresadola (Ann. Mycol. **1**: 115. 1903) based *Eichleriella* on more or less arid forms, often with free margins. Patouillard (Tax. Hymen. 24. 1900) had previously separated such forms in his section *Hirneolina* of *Sebacina* and Saccardo (Syll. Fung. **17**: 208. 1905) raised this to generic rank. *Hirneolina* has been widely used, but, as a genus, it is clearly a later synonym of *Eichleriella*. Bourdot (Trans. British Mycol. Soc. **7**: 53. 1920) established the subgenus *Bourdotia* for a species of *Sebacina* with gloeocystidia. This was later raised by Bresadola and Torrend (Brotéria ser. bot. **11**: 88. 1913) to generic rank.[3]

Rea (British Basid. 737–738. 1922) treated *Bourdotia* and *Heterochaetella* as subgenera of *Sebacina* and proposed the subgenus *Eusebacina* to include the remaining species. Bourdot and Galzin (Hymén. Fr. 35–53. 1928) recognized *Bourdotia, Heterochaetella* and *Sebacina* as coordinate genera. Rogers (Univ. Iowa Stud. Nat. Hist. **15**(3): 9–13. 1933) argued against this on the ground that the groups tend to grade into each other, but admitted that they make convenient groupings and may be treated as subgenera. This was followed by McGuire (Lloydia **4**: 1–43. 1941), who called them sections. Martin's treatments of the genus (Univ. Iowa Stud. Nat. Hist. **18**(3): 37–46. 1944; **19**(3): 44–61. 1952) are based on McGuire's work. The genus, as presented by McGuire and by Martin, is heterogeneous, the assigned species having in common, in addition to the tremellaceous basidia, only a resupinate habit and a smooth or undulate hymenium.

Evidence other than the presence or absence of gloeocystidia or seta-like cystidia for the elevation of the sections of *Sebacina*, as defined by McGuire, to generic rank may be found by a study of microtome sections through the fructifications, and of free-hand sections pressed out to show the arrangement and development of the basidia. The species included in McGuire's section *Eusebacina* tend to have definite hymenial layers and, in well-developed collections, often display two or more strata, as evidenced by differences in thickness or denseness of hyphae, almost certainly representing a response to rain after a dry interval.

Sebacina (*Heterochaetella*) *dubia* is characterized by long, bristle-

[3] In the reference cited, the authors described an additional species as *Bourdotia caesia*. They did not specifically say they were raising the subgenus to the rank of a genus, but they treated it as a genus and compared it with the previous species, using the binomial *Bourdotia galzinii* Bres. It seems permissible to recognize this as sufficient indication that they intended to raise *Bourdotia* to generic rank. In any event, the generic status was clearly recognized by Saccardo (Syll. Fung. **23**: 571. 1925) and by Bourdot and Galzin (Hymén. Fr. 48. 1928).

like cystidia which emerge from a dense horizontal layer of hyphae on the surface of wood permeated by hyphae. These cystidia are entirely distinct from hymenial structures known elsewhere in the Tremellaceae. The dense basal layer gives rise to a loose, floccose zone of hyphae amongst which the basidia are rather sparsely distributed. The basidia are ovoid, with extremely short epibasidia, scarcely distinguishable from the sterigmata. This species has little in common with *Sebacina* aside from its resupinate habit and cruciate-septate basidia. The generic status given to *Heterochaetella* by Bourdot and Galzin is fully justified.

The hymenial layer in those species included in the section *Bourdotia* tends to be less definite, with stratification lacking or obscure except in a few tropical collections examined. Three or four layers of horizontal hyphae may make up the basal layer next to the substratum; gloeocystidia and basidia usually occur throughout the remainder of the fructification. In addition to the gloeocystidia, the basis on which Bresadola gave *Bourdotia* subgeneric status, the development of the basidia, as pointed out by Rogers (1933), is, in several species, very different from that of other species included in *Sebacina.* Examination of a teased-out freehand section of such a species shows stalk-like hyphae bearing near their tips turgid developing and mature basidia, while the empty and collapsed older basidia form characteristic involucres surrounding the older portions of the stalks. This method of producing basidia is not evident in *B. galzinii,* which must be the type, and it may be that another genus will some time be regarded as necessary for the species with the characteristic mentioned. For the present, and to avoid premature name-changing, it seems best to retain the resupinate gloeocystidiate species in *Bourdotia,* where they were placed by Bourdot and Galzin. The erumpent gelatinous form, *Bourdotia pululahuana* (Pat.) Bourd. & Galz., based on *Tremella pululahuana* Pat., has previously been excluded from *Bourdotia* because of its non-resupinate habit and the different basidial arrangement (Ervin, Mycologia **48**: 690–693. 1956).

The rest of the species of *Sebacina,* in the wide sense, may be separated into two groups on the basis of their consistency and of the presence or absence of clamp-connections. One group is composed of thick-gelatinous to tough-coriaceous forms without clamp-connections, and includes *Sebacina incrustans.* For this group, the generic name *Sebacina* must be retained. The second group is composed of waxy-gelatinous forms which dry to a thin, often inconspicuous, film, and possess clamp-connections. For these, the generic name *Exidiopsis* appears to be available.

Many years ago, Brefeld (Unters. **7**: 94. 1888) described *Exidiopsis* as a subgenus of *Exidia*, based on *Exidia* (*Exidiopsis*) *effusa*. This was a broadly effused, *Corticium*-like fungus of waxy-gelatinous consistency. Möller (Protobasid. 82. 1895) raised this to a genus, describing five species. Other authors have added additional species, not always with discrimination, so that 16 or more combinations have been made in the genus. Whether the type of *Exidia effusa* is still in existence is uncertain, but Brefeld's description, and particularly his Pl. 5, figs. 20–22, make it certain that the species upon which *Exidiopsis* was based is a typical member of this group. Bourdot and Galzin (Hymén. Fr. 44. 1928) cite *Exidiopsis effusa* Bref. as a synonym of *Sebacina uvida* (Fr.) Bres. and retain *Exidiopsis* as a section of *Sebacina*. In my opinion, these species should be segregated from *Sebacina incrustans* and its associated species, and grouped as the genus *Exidiopsis*.

Thus, four genera may be derived from *Sebacina* as treated by McGuire and by Martin. A key to these genera, generic diagnoses, and mention of included species follows:

a. Bristle-like cystidia with apically dilated lumina present; gloeocystidia lacking...*Heterochaetella*
a. Cystidia lacking as a rule, when present, few and not bristle-like..............b
  b. Gloeocystidia present, their contents finally yellow or brown.....*Bourdotia*
  b. Gloeocystidia lacking..c
    c. Tough-coriaceous to gelatinous; clamp-connections lacking..*Sebacina*
    c. Waxy-gelatinous to subarid; clamp-connections present....*Exidiopsis*

HETEROCHAETELLA (Bourd.) Bourd. & Galz., Hymén. Fr. 51. 1928.
  Sebacina subg. *Heterochaetella* Bourd, Trans. British Mycol. Soc. **7**: 53. 1921.[4]

Resupinate, effused, firm-gelatinous to mucous, velvety; cystidia bristle-like, firm, long-emergent, with apically dilated lumina, numerous; probasidia ovoid; basidia cruciate-septate, with short, subulate epibasidia merging into sterigmata.

Type: *Heterochaete dubia* Bourd. & Galz., Bull. Soc. Myc. Fr. **25**: 30. 1909.

[4] Bourdot's subgenus *Heterochaetella* was established in connection with his description of what he regarded as a second species, [*Sebacina*] *Heterochaetella crystallina* Bourd. Bourdot specifically says: "This new subgenus *Heterochaetella* till now only included *H. dubia* with allied forms or species which have not yet been published . . ." clearly implying that *H. dubia* is the type. This was recognized by McGuire (Lloydia **4**: 1. 1941) as indication of the type. The first formal publication of the combination *Sebacina dubia* (Bourd. & Galz.) Bourd. appears in Assn. Fr. Av. Sci. **45**: 576. 1922. It is doubtful whether *H. crystallina* belongs in *Heterochaetella* as here defined.

SEBACINA Tul., Jour. Linn. Soc. Bot. **13**: 35. 1871.

Resupinate or incrusting, sometimes with free lobes, usually thick; texture tough, coriaceous or gelatinous; hymenium smooth or undulate; probasidia subglobose or ovate, becoming cruciate-septate, each cell so formed developing a tubular epibasidium which may become long and tortuous; gloeocystidia lacking; clamp-connections lacking; basidiospores white in mass, germinating by repetition or by the production of conidia.

Type: *Corticium incrustans* Pers.

The following species were examined: *S. incrustans* (Pers. ex Fries) Tul.; *S. helvelloides* (Schw.) Burt; *S. epigaea* (Berk. & Br.) Rea.

BOURDOTIA (Bres.) Bres. & Torrend, in Torrend. Brotéria ser. bot. **11**: 88. 1913.

Sebacina subg. *Bourdotia* Bres., Ann. Mycol. **6**: 46. 1908.[5]

Resupinate, effused, arid-waxy to waxy-gelatinous, usually pallid but sometimes drying to a brown or blackish crust; hymenial region consisting of numerous gloeocystidia and penetrated by erect fertile hyphae bearing the basidia terminally and proliferating, the older hyphae often sheathed by the remains of the collapsed basidia; basidia globose to subglobose, longitudinally cruciate-septate, each basidial cell producing an epibasidium on which the basidiospore is borne on a short sterigma; basidiospores germinating by repetition or by the production of conidia.

Type: *Sebacina* (*Bourdotia*) *galzinii* Bres.

The following species, here included in *Bourdotia*, were examined: *B. cinerea* (Bres.) Bourd. & Galz.; *B. deminuta* (Bourd.) Bourd. & Galz.; *B. eyrei* (Wakef.) Bourd. & Galz.; *B. caesio-cinerea* (Höhn. & Litsch.) Bourd. & Galz.; *B. galzinii* (Bres.) Bres. & Torr.

EXIDIOPSIS (Bref.) Möller, Protobasid. 167. 1895.

Exidia subg. *Exidiopsis* Brefeld, Untersuch. **7**: 94. 1888.

Resupinate, waxy-gelatinous to subarid, drying to an inconspicuous film or a dark crust; hymenium smooth or undulate, two or more successive hymenial layers often present; probasidia subglobose or ovate, becoming cruciate-septate, each cell developing a tubular epibasidium, usually relatively long and sometimes tortuous; gloeocystidia lacking; clamp-connections present; basidiospores germinating by repetition or by the production of conidia.

[5] Bourdot and Galzin treated both *B. galzinii*, the type species, and *B. caesia* as subspecies of what they called *B. pululahuana*. I regard them as quite distinct. See Mycologia **48**: 690–691. 1956, also footnote 3.

Type species: *Exidia (Exidiopsis) effusa* Bref.

The following species were examined and are transferred to *Exidiopsis:* **E. podlachica** (Bres.) comb. nov., based on *Sebacina podlachica* Bres., Ann. Mycol. **1**: 117. 1903; **E. molybdea** (McGuire) comb. nov., based on *Sebacina molybdea* McGuire, Lloydia **4**: 17. 1941; **E. fugacissima** (Bourt. & Galz.) comb. nov., based on *Sebacina fugacissima* Bourd. & Galz., Bull. Soc. Myc. Fr. **25**: 28. 1909; **E. prolifera** (Rogers) comb. nov., based on *Sebacina prolifera* Rogers, Mycologia **28**: 350. 1936; **E. sublilacina** (Martin) comb. nov., based on *Sebacina sublilacina* Martin, Mycologia **26**: 262. 1934.

This work was done under the direction of Professor G. W. Martin. I am indebted to Dr. Donald P. Rogers and Mr. Kenneth Wells for a number of corrections and suggestions.

STATE UNIVERSITY OF IOWA,
IOWA CITY, IOWA

MYROTHECIUM RORIDUM ON GARDENIA [1]

CHARLES L. FERGUS

(WITH 2 FIGURES)

In the summer of 1952, an apparently new leaf spot disease of gardenia was collected by Mr. G. B. Sleesman, a state nursery inspector, in several greenhouses in southeastern Pennsylvania. The specimens were communicated to the author for identification by Dr. O. D. Burke, of the Department of Plant Pathology Extension of the Pennsylvania State University.

The sporodochia of *Myrothecium roridum* Tode ex Fries (**4**) were found on the lower surfaces of many of the infected leaves. The pathogenicity of *M. roridum* has been the subject of a number of publications (**3**, **5**, **6**, **7**, **8**). It is a proven pathogen for pansy, violet, snapdragon, cowpea, soybean and tomato. It has also been collected from many kinds of decaying plant tissues (**5**) as well as from soils (**9**).

Host indices do not list this fungus as a pathogen of gardenia. However, a thorough survey of the literature revealed two abstracts (**1**, **2**) which reported collections of the fungus on gardenia. Because of the lack of illustrations and incomplete description of symptoms and signs, the disease was studied more fully.

The leaf spots are circular at first, enlarging progressively, and in most cases remaining circular. However, under extremely moist conditions the affected area may be very irregular and quite extensive. Vein tissue is included in the necrosis. The circular spots vary in size up to 2 cm in diameter. Occasionally the center of the lesion cracks irregularly and ruptures, in which case some leaf tissue may drop out. Newly invaded tissue appears water-soaked at first, later becoming brown. The center of the spot may be light brown, while the advancing edges are darker. The spot on the upper leaf surface is usually darker than on the lower leaf surface, becoming practically black. The leaf symptoms are presented in FIG. 1.

Sporodochia form on both surfaces of the leaf, but are produced more abundantly on the lower surface. See lower left FIG. 1. Usually

[1] Contribution No. 212 of the Department of Botany and Plant Pathology. Authorized for publication August 3, 1956, as paper No. 2080 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

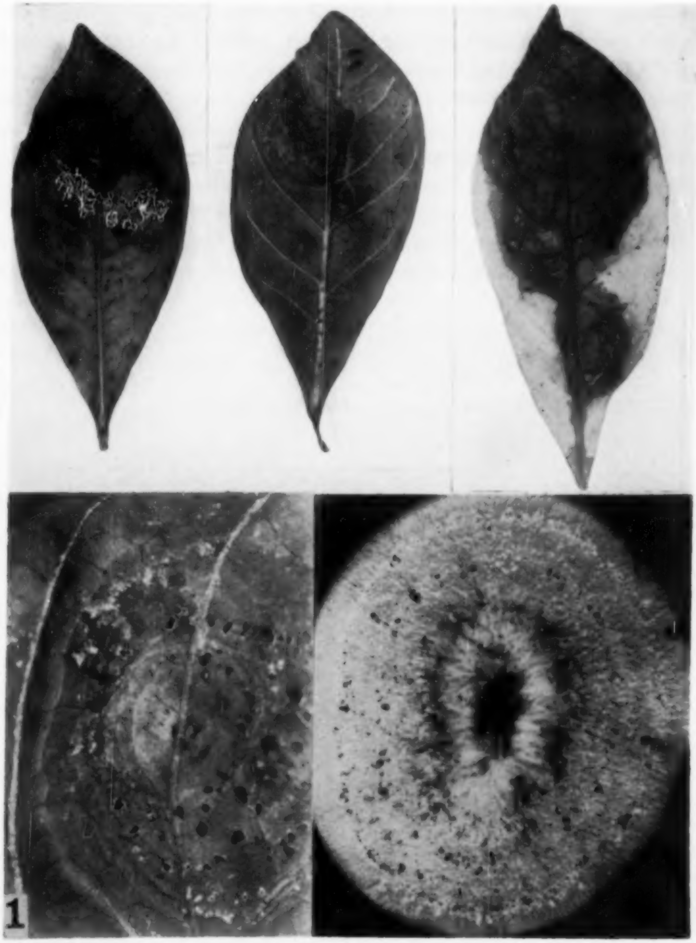


FIG. 1. Upper, leaves of gardenia showing leaf spots. Lower left, white-rimmed sporodochia of *M. roridum*, and right, pure culture on malt extract agar.

they are not formed in any definite manner, but occasionally a circular pattern may be observed. The sporodochia are small, dark green or black masses surrounded by distinct white setal margins. They are circular, discoid or irregular in shape, and vary from 1 to 2 mm in diameter. Several may coalesce into a larger mass. FIG. 2 illustrates median sections through several sporodochia.

The sporodochia are covered with a watery-viscid spore mass which is dark green at first, becoming jet black. Upon drying they become dull black. The conidia are hyaline or slightly tinted, straight with rounded ends, and 1-celled. Mounted in lactophenol they measured $4.5–8 \times 1.5–2\,\mu$. They are borne on slenderly clavate phialides, which form as closely appressed whorls at the apex of a septate, hyaline conidiophore. The conidiophores arise from a pseudoparenchymatous base which is variable in thickness (FIG. 2).

Pure cultures were obtained by streaking spores on malt extract agar and also by tissue isolation following chlorox sterilization.

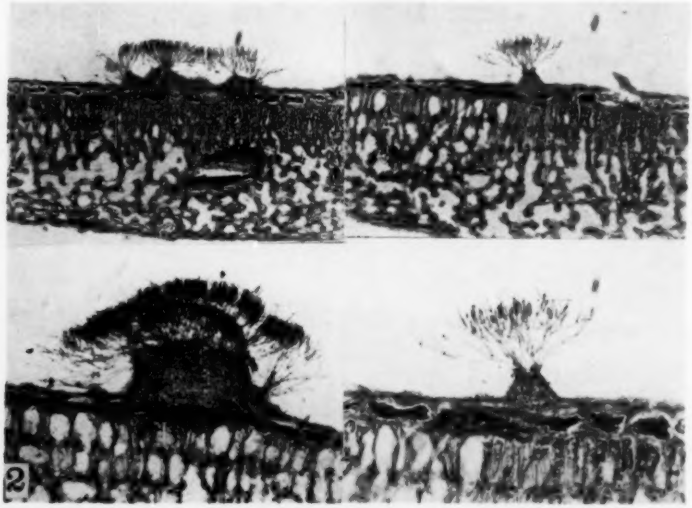


FIG. 2. Median sections through sporodochia of *M. roridum* on gardenia leaves.

The fungus grows well on various artificial media, including malt extract, Czapek-Dox, potato dextrose, corn meal, and prune agars. It produces a floccose growth of white hyphae with scattered black sporodochia (see lower right FIG. 1).

It is relatively tolerant of pH on malt extract agar (adjusted with 0.2N NaOH or HCl), germinating and growing well over a wide range. Colony diameter (the average of three petri dish cultures) at various pH values from 4.8 to 10.6 after 9 days growth was 35 mm, and germination (after 18 hours) averaged 90% or more. At pH 3.6, less than 1% of the spores germinated and the colony attained a diameter of only 8 mm. No spores germinated and no growth occurred at 2.7.

The relation of temperature to growth and germination was studied on malt extract agar. After 14 days incubation at 6° C, the colony diameter was 8 mm and dry weight was 18 mg; at 12° C, 20 mm, 30 mg; at 18° C, 52 mm, 134 mg; at 25° C, 82 mm, 341 mg; at 30° C, 85 mm, 235 mg; and at 35° C, 26 mm, 88 mg. Spore germination of 95% was observed at 25 and 30° C, 57% at 18° C, and less than 1% at 6° C.

Artificial inoculations of leaves of gardenia failed unless the leaves were previously wounded. This directly contradicts the results of Barrett (**1**), who reported establishing its pathogenicity with successful infection occurring in the absence of wounds. It should be noted that Brooks (**3**) reported variation in pathogenicity among isolates of *M. roridum,* one isolate from potato being non-pathogenic to potato.

M. roridum is apparently a wide-spread saprogen capable of invading plants that have been wounded or exposed to certain environmental conditions, such as excessive humidity. Barrett and Hardman (**2**) reported it as the cause of a disease of gardenia cuttings in rooting beds under very humid and warm conditions. Being widely distributed and with a variability in pathogenicity, the host range may be discovered in the future to be much wider than now known.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY,
PENNSYLVANIA STATE UNIVERSITY,
UNIVERSITY PARK, PENNSYLVANIA

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THE MYXOMYCETES OF THE MUSSOORIE HILLS. IV

G. W. Martin, K. S. Thind and H. S. Sohi

(with 3 figures)

Under the leadership of Professor P. N. Meshra, an excursion is made every year from the Botany Department of the Panjab University to the Mussoorie Hills in the northwest Himalayas (alt. 5000–7000 ft.), to study the cryptogams. The study of Myxomycetes by Dr. K. S. Thind and his students is a part of the program. The first three contributions (**2**, **3**, **4**) give accounts of 22 known species; the three new species here described constitute Nos. 23–25 of this series. The classification of Martin (**1**) has been followed throughout.

23. **Stemonitis mussooriensis** sp. nov. Fig. 1

Sporangia cylindrica, stipitata, nigra, ad 3 mm alta, gregaria in hypothallo argente; stipes brevis, erectus, $\frac{1}{4}$ vel $\frac{1}{3}$ totius altus; columello brunneo apicem peridii subattingente, sursum plerumque flexus producto; capillitio ex floccis gracilibus, brunneis, flexuosis composito, ramis anastomosantibus; masculis superficialibus magnis, irregularibus, ad 130 μ diam.; sporis globosis, verrucosis, brunneis, 10.5–12.5 μ diam.

Sporangia cylindrical, stipitate, short, black, up to 3 mm in height and 0.6–0.7 mm wide, erect or sometimes curved, with obtuse apices, crowded in large tufts on a shining, silvery hypothallus; stipe short, erect, jet black, $\frac{1}{4}$ to $\frac{1}{3}$ the total height of the sporangium, expanded at base, gradually tapering upward; peridium evanescent, irregularly dehiscent; columella a prolongation of the stipe, prominent, thick, central, black, unbranched, tapering gradually upward as it gives rise to the capillitium, flexuous above, ending abruptly just below the obtuse sporangial apex; columella lax, composed of branching, tapering and anastomosing violaceous brown threads, often expanded and paler at points of union, the meshes irregular, up to 130 μ in diameter, the ultimate branchlets slender and anastomosing to form a surface net of smaller meshes than those of the interior; spores black in mass, violaceous brown by transmitted light, profusely and prominently warted, 10.5–12.5 μ in diameter including the slender warts which may be as much as 0.8 μ in height.

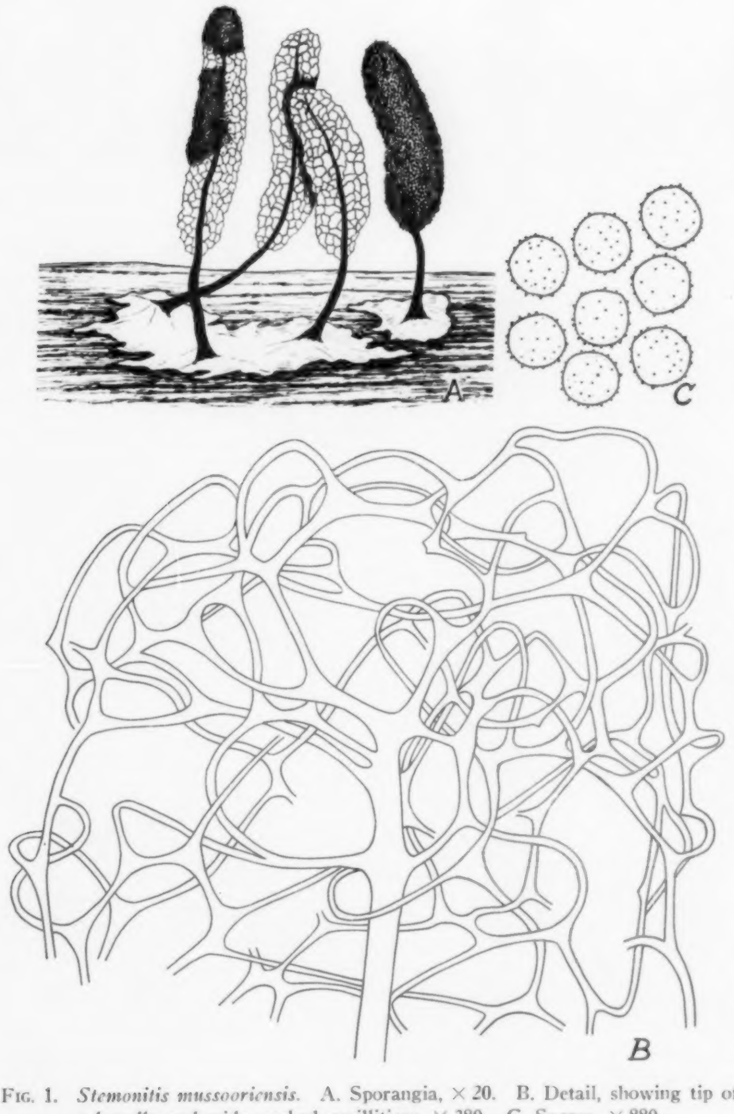

FIG. 1. *Stemonitis mussooriensis.* A. Sporangia, × 20. B. Detail, showing tip of columella and wide-meshed capillitium, × 380. C. Spores, × 880.

INDIA: Jamna Bridge, Mussoorie, 30 Aug. 1950, on decayed bark. No. *86*, TYPE.

This species is closely allied to *Stemonitis webberi* Rex, from which it differs in the very short sporangia (2–3 mm as compared with 5–15 mm in *webberi*), in the much larger spores (10.5–12.5 μ as compared with 8–9 μ in *webberi*), in the more conspicuous warts on the spores,

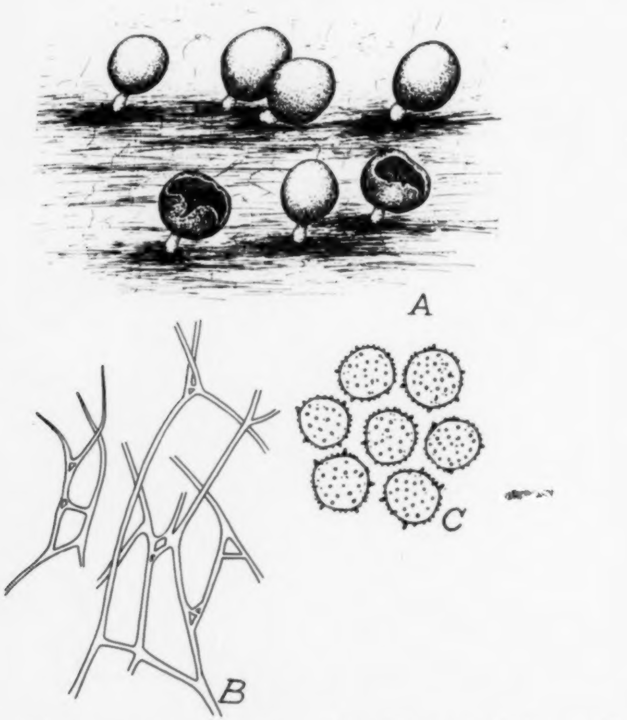

FIG. 2. *Lamproderma verrucosum.* A. Sporangia, × 20. B. Capillitium, × 380. C. Spores, × 880.

and in the lack of any trace of the coppery iridescence of the capillitium characteristic of *webberi*.

24. **Lamproderma verrucosum** sp. nov. FIG. 2

Sporangia gregaria, globosa, stipitata, 0.3–0.4 mm diam., atro-cyanea vel atro-violacea, iridescentia; stipites brevissimi, crassi, aurantiaci; peridio membranaceo pallide purpureo; columella brevi; capillitiis e filamentis densis, ramosis, apicibus

pallidis composito; sporis fusco-brunneis prominenter et irregulariter verrucosis, (9–)10–11 μ diam.

Sporangia gregarious or scattered, up to 0.4 mm in diameter, navy blue to deep violet, iridescent with brilliant metallic luster; stipe orange, short, thick, stout, longitudially furrowed, tapering upward; hypothallus brown, well-developed; peridium persistent, membranous, wrinkled, dehiscing irregularly from above; columella very short; capillitium dense, rigid, the threads branching and anastomosing freely, deep violet, becoming paler at the tips; spores black in mass, dark violet-brown by transmitted light, globose, or occasionally broadly elliptical, coarsely, irregularly and sparsely, but prominently verrucose, 9–11 μ in diameter, including the warts, which may be up to 1 μ long.

INDIA: The Company Garden, Mussoorie, 22 August, 1952, on leaves of *Quercus incana*. No. *87*, TYPE.

This species differs from previously described species of *Lamproderma* in its very short columella, its short orange stalk and in the coarsely and sparsely verrucose spores. *L. muscorum* (Lév.) Hagelst., which also occurs on leaves, is larger, with a black stalk, a well-developed columella, and slightly larger spores with smaller, less prominent spines.

25. **Tubifera papillata** sp. nov. FIG. 3

Sporangia cylindrica, ad 3 mm alta, 0.7 mm crassa, peridiis membranaceis, nitentibus, in caput brunneum subglobosum 6–7 mm latum conglomerata; hypothallo cylindrico, sulcato, 3 mm altitudine, quasi stipitem formante; massa sporarum ferruginea; sporis pallide brunneis, reticulato-papillatis, 6.5–7.7 μ.

Sporangia cylindrical, narrowed at the obtuse apex, up to 3 mm tall and 0.7 mm wide, densely crowded but only slightly angular, forming a pseudoaethalium up to 7 mm broad, borne on the stipe-like hypothallus; peridium thin, membranous, shining, rupturing at the apex, the lower portion persistent; hypothallus columnar, dark brown, up to 3 mm in height; spores uniformly papillate, with a very faint underlying, somewhat irregular reticulation, 6.5–7.7 μ.

INDIA: Kempty Fall, Mussoorie, 15 August 1952, on rotten wood. No. *88*, TYPE.

Resembling *T. microsperma* (Berk. & Curt.) Martin in its stalk-like hypothallus, but differing in the more loosely aggregated sporangia and the larger papillate spores with underlying reticulation so faint as to be scarcely visible under an oil immersion objective unless the spores are stained.

The type collections have been deposited in the herbarium of the Panjab University, Amritsar, with portions of them in the herbarium of the State University of Iowa.

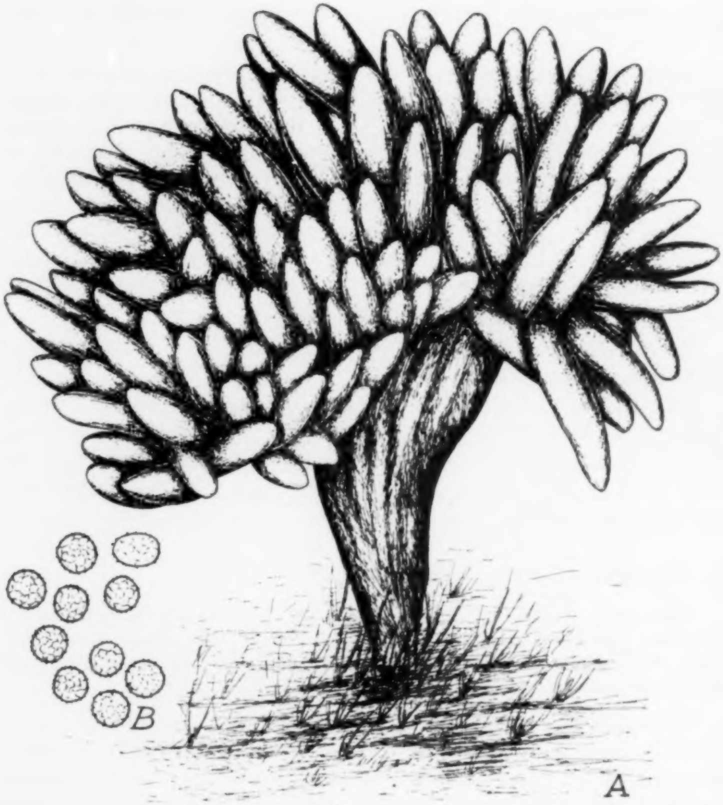

FIG. 3. *Tubifera papillata.* A. Sporangial cluster on stipe-like hypothallus, × 10. B. Spores, showing papillate surface and faint reticulation, × 88.

The junior authors are indebted to Professor P. N. Meshra for encouragement and facilities and to Mr. B. Khanna for assistance in the preparation of the illustrations.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS
AND
DEPARTMENT OF BOTANY,
PANJAB UNIVERSITY,
AMRITSAR, PANJAB, INDIA

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CHARLES THOM [1]
1872–1956

Kenneth B. Raper

(with portrait)

With the death of Dr. Charles Thom at his home in Port Jefferson, New York, on May 24, 1956, the Mycological Society of America lost one of its most colorful and most productive members. For more than half a century he was a consistent contributor to mycological literature, publishing many of his researches and addresses in the Society's journal, Mycologia. Although he was best known to mycologists for his monumental studies on *Aspergillus* and *Penicillium*, he was a scientist with exceptionally wide interests and experience who was equally at home in the dairy, the canning factory and the cotton field. His interests in all biology stemmed naturally from his early contacts with many outstanding biologists who implanted in him a thirst for discovery and scholarly pursuits which never waned. His broad experience resulted in part from the various official positions which he held during his long career in the United States Department of Agriculture (1904–1942), but more particularly from an exceptional intellect and an insatiable curiosity that was applied to every assignment. He grew up in an area just removed from the Frontier where a good education was not obtained without sacrifice, hence was highly prized. He early learned the true value of long hours of work, of mental and physical discipline and of independence in thought and action, and he unrelentingly marshalled these attributes to fashion a professional career with many facets, yet singularly devoted to scholarship and public service.

Charles Thom was born on a farm just north of Minonk, Illinois, on November 11, 1872. He was the fifth of six sons (two died in infancy) of Angus Sutherland Thom, who as a boy of sixteen had

[1] The preparation of this memoir has been greatly facilitated by autobiographical notes compiled by Dr. Thom and by personal papers, reprints, and correspondence made available to me by his family following Dr. Thom's death. In a single instance his words are paraphrased from memory; otherwise the quotations are taken from the above material or from his publications. Technical reports and addresses distributed by the U. S. Department of Agriculture only in mimeograph form are omitted from the list of publications.

CHARLES THOM
Gainesville, Florida, January 30, 1950.

moved in 1848 with his family by wagon train from Ohio. His mother, Louisa Electa Herrick, arrived in Illinois eight years later with her family from Indiana, having moved there in 1838 from Northern Virginia. The Thoms were, as Dr. Thom often related, of Scotch-Irish ancestry and "persistently Presbyterian"; his mother was of English and Quaker descent. Neither of his parents possessed much formal schooling, but they were people of scholarly instinct who were determined to secure for their sons the education that they had been denied. Dr. Thom's mother taught him to read and gave him elementary instruction in arithmetic, geography and grammar before he entered public school at the age of eight. The local high school fell short of college preparation and in 1889 he entered Lake Forest Academy (Illinois) where he came under the influence of R. A. Harper, then an instructor. He enrolled in Lake Forest College two years later. These were years of exceptional inspiration which exerted a profound and lasting influence upon his life and work. Harper was teaching botany, Wm. A. Locy was professor of zoology, and in Thom's junior year John M. Coulter became president of this small college. After graduation he spent one year teaching high school in Danville, Illinois, but returned to Lake Forest in 1896 and became the first graduate student to take an advanced degree (M.A., 1897) with Harper. The summer of 1897 was spent at Woods Hole before going to the University of Missouri where he was both instructor and graduate student. There he worked with Howard Ayers who further kindled his enthusiasm for biological research. He received his doctorate in 1899 with a dissertation on "The process of fertilization in *Aspidium* and *Adiantum*," his being the first Ph.D. degree to be awarded by that institution. He remained at Missouri for three more years, teaching botany during the school year and spending his summers elsewhere. One of these was spent collecting plants in the Ozark Mountains of Missouri and another in the swamps of southeast Missouri; the third found him in Erwin F. Smith's laboratory in Washington, D. C. An interest in the fungi engendered by Harper was greatly enhanced by these experiences and in the summer of 1902 he moved to Cornell University as a graduate assistant in mycology with George F. Atkinson. B. M. Duggar and H. H. Whetzel were there at the same time, and in company with these eminent mycologists-to-be his dedication to this field of study became firmly established. That his life's work should follow the course which it did was determined by a circumstance which, in characteristic fashion, he relates as follows:

"In February, 1904, Professor Atkinson was asked to name a dairy mycologist for a project in cheese ripening. He looked around his laboratory and fixed on me to be sent, rather peremptorily offered me the nomination, then wrote a letter. I saw that letter long afterward. He wrote approximately this: 'I know no man in America that is qualified in Dairy Mycology. I know nothing about it myself but if I had time, I could learn it, but I am too busy. Thom has training enough and brains enough to learn it and he needs the job.' I resigned my petty but perfectly orthodox academic job at Cornell and went." [2]

The job was with H. W. Conn of the Storrs (Connecticut) Experiment Station where a project had been initiated on mold-ripened cheeses. Thom soon found his assignment to be far more than straight mycology, for while a specific mold was central to the production of a particular type of cheese the problems of manufacture were much more involved. Thus he became an applied microbiologist, but one who never lost sight of the essentiality of thorough, painstaking taxonomic research, and it was in this latter area that he made what will probably prove to be his most lasting contributions to science. In beginning his work at Storrs he first isolated the molds from imported Camembert and Roquefort cheeses—which he was expected to duplicate and manufacture in the United States. He turned to the then existing mycological literature and found a plethora of latin names but without agreement as to usage and without descriptions adequate to reveal the characteristics of the molds to which they had been applied. Thus, in 1906, he was led to describe *Penicillium camemberti* and *P. roqueforti.* For purposes of comparison he had isolated a dozen or more other Penicillia, and being equally unable to relate these satisfactorily to the older literature, he began his monographic studies on this ubiquitous genus. These studies brought him in frequent contact with W. G. Farlow and Roland Thaxter at Harvard, and it was the latter who urged him to "clean up the mess in *Penicillium* and *Aspergillus.*"

The first important step in this direction was taken in 1910 with the publication of his "Cultural Studies of Species of Penicillium." In this he renounced the prevailing practice of describing members of this genus from their natural habitats and turned to defined, reproducible media and the comparative examination of cultures grown in the laboratory. The soundness of this approach is witnessed by the fact that nearly a half-century later virtually all of the species described in 1910 are still

[2] From "Molds, mutants and monographers." *Mycologia* **44**: 61–62. 1952.

recognized as valid taxons. The next step came in 1915 with the publication of "The *Penicillium luteum-purpurogenum* group," for in this brief paper he advanced the notion that the assignment of molds possessing common basic characteristics to groups and series was, for most practical purposes, of greater importance than identification to species. The merit of this concept has been amply demonstrated many times but nowhere more forcefully than in the search for superior penicillin-producing molds during World War II.

Dr. Thom's work at Storrs was singularly successful, for he succeeded in introducing practical processes for the manufacture of Camembert and Roquefort cheeses in the United States before his work was moved to Washington, D. C., in 1913. Visualizing an opportunity for greater service, he accepted the following year the position of Mycologist in Charge of the Microbiological Laboratory, Bureau of Chemistry, which was charged with the enforcement of certain aspects of the Pure Food and Drug Act enacted by the Congress eight years earlier. Thom took the position that the criteria for judging the quality of foods and the approval of processing methods should extend beyond actual toxicity and danger to health. He maintained that "sight, taste and smell were given to man to keep him from eating rotten food, and that decomposed and heavily contaminated materials were intrinsically offensive and always bore evidence of mishandling." The courts supported his position, and the confidence with which we today unthinkingly purchase canned or otherwise processed foods from our grocer's shelf is a silent tribute to the effectiveness of the campaign waged by Thom and his associates. From conversations with him years later, I doubt that he was ever happier than when arguing a case in court. (He would have made an excellent prosecuting attorney.) One of many antecdotes will illustrate the ingenuity of his approach—the case involved tomato catsup: As he took the witness stand Thom quietly placed on the railing of the jury box two partially rotten tomatoes. Naturally, these were regarded with considerable curiosity. Upon leaving the stand as he finished testifying, he pointed to them and asked, "Should the American people have to eat them in their catsup?" The jury agreed. Thom remained in this position until 1927, and the principles evolved and established for the food industries were summarized in the book, *Hygienic Fundamentals of Food Handling,* published with A. C. Hunter in 1924.

Mycologically, these were very productive years. James N. Currie, chemist, had followed Thom from the Cheese Investigation, and he was now joined by the mycologist, Margaret B. Church. Molds were encountered at every step in the harvesting, handling and processing of

many foods, and, as in the cheese laboratory and factory, most prominent among these were species of *Aspergillus* and *Penicillium*. With Miss Church he published a series of papers on different groups of the Aspergilli, further emphasizing the primary importance of the group characteristics in these fungi. These studies culminated in the publication of their monograph, *The Aspergilli,* in 1926. Meanwhile, Thom and Currie had given particular attention to the black-spored species comprising the *Aspergillus niger* group. They had early detected unusual metabolic activity in these fungi and in 1916–17 demonstrated that certain of these molds if cultivated under appropriate conditions would produce very substantial amounts of citric acid. Within five years a large factory had been constructed and the first major mold fermentation had become firmly established. With Thom's collaboration, other investigators in a different branch of the Department of Agriculture (H. T. Herrick, O. E. May, P. W. Wells, A. J. Moyer and L. B. Lockwood) subsequently took up this line of investigation to extend the still lengthening list of such industrial fermentations.

From 1927 until his retirement in 1942, Dr. Thom was Mycologist in Charge of the Division of Soil Microbiology, first in the Bureau of Chemistry and Soils and after 1934 in the Bureau of Plant Industry. Concurrent with his investigations of the Aspergilli, he continued his taxonomic studies of *Penicillium*. Because of the larger dimensions of this genus, its more voluminous literature, and the greater difficulties encountered in differentiating groups, series and the species which comprised these, publication of his monograph, *The Penicillia,* was delayed until 1929. This new book, like the one on *Aspergillus* before it, immediately achieved universal recognition as the standard reference work on *Penicillium*. As Dr. Thom moved from one official assignment to the next he took his growing culture collection with him, for he was never satisfied to base decisions upon published descriptions alone if there was any possibility of obtaining living cultures of the actual molds studied by his predecessors. In this he was highly successful, and when I joined him in 1929 to assume the responsibility for their maintenance, these accessions numbered well over one thousand, including scores of types. The reader will readily understand why these cultures later became the nucleus of the Culture Collection which the U. S. Department of Agriculture subsequently instituted at its Northern Regional Laboratory in Peoria, Illinois, and why the writer, as Thom's protégé, was given the opportunity of organizing and directing that collection. It is most fortunate for mycology and for applied microbiology that, for nearly four decades, Thom had the vision and the energy to carry

forward his taxonomic investigations on *Aspergillus* and *Penicillium* over and above his assigned duties as the responsible scientist in each of his succeeding positions. The handsome dividends resulting from his selfless devotion to this ideal are today self-evident.

Dr. Thom retired from active service in the Department of Agriculture in 1942 at age seventy, but in the years immediately preceding this I had joined him in the publication of researches on the *Aspergillus nidulans* and *A. glaucus* groups. A considerable number of new forms had been isolated in our laboratory and elsewhere and it was realized that those sections of his earlier monograph did not adequately present the ascosporic members of these two groups. By 1943 it was evident that similar treatment should be accorded other sections of the genus as well and, with Thom then a collaborator to the Northern Laboratory, we published the *Monograph of the Aspergilli* in 1945. Four years later, with financial support from the National Science Fund and with the invaluable aid of Dorthy I. Fennell, a completely rewritten volume on *Penicillium* was published under the comparable title, *Manual of the Penicillia*. Thom's interest in these fungi never diminished, and his bibliography reveals that one of his last contributions to science, published in 1954, concerned itself with the evolution of species concepts in these two singularly important genera of molds. Thus, a study modestly begun by a young man groping for an understanding of two molds that he had isolated from cheese gradually developed into a lifetime of productive research with incalculable benefit to all mankind. Whereas these investigations were important in themselves and stand as models of what should be done for scores of other fungus genera, their greater importance perhaps lies in the new and enlarged dimensions they created for all of microbiology. It is no exaggeration to say that the current stature of the antibiotics industry can be traced directly to the early researches of Thom and his associates.

Two inadequately publicized incidents may be cited in support of this notion. Fleming had discovered penicillin in 1928 and had published his classic paper in 1929, identifying the responsible mold as "*Penicillium rubrum*." Harold Raistrick, who had long collaborated with Thom, subsequently took up the study of this mold and its antibacterial substance. He submitted the culture to Thom who correctly identified it as representative of the cosmopolitan species, *P. notatum* Westling. Later, when more productive strains were urgently needed, this correct diagnosis gave invaluable direction to our quest for such cultures. Equally important, it was Thom who recommended that our Government's research program on penicillin be centered in the newly

established Northern Regional Research Laboratory in Peoria. Had Thom, the world's recognized authority on *Penicillium*, been a less unselfish man he might easily have suggested that this work be placed in his laboratory at Beltsville, and in so doing possibly have delayed his impending retirement from the U. S. Department of Agriculture. For reasons which he could evaluate better than anyone else he made his decision unhesitatingly. If Dr. Thom ever felt that he should have received greater recognition for his contribution to the success of this program he never expressed this publicly.

Charles Thom was a pioneer in other areas as well. In collaboration with Harry S. Bernton, M.D., of Washington he was one of the first to demonstrate the role of common saprophytic fungi as the incitants of allergic reactions in man. Due in part to this and because of his earlier role as a Government official charged with the responsibility of establishing sanitary practices in the nation's food industries he was invited to become a member of the Washington Academy of Medicine, a unique tribute to a non-medical man.

In 1934, with Harry Humfeld and H. P. Holman he was instrumental in developing the "*Chaetomium* Test" for the evaluation of fungicides and other protectants used to extend the usefulness of awnings, tarpaulins, etc. This was designed to provide at least a partially quantitative test, and although it has now been supplemented and in substantial part superseded by other methods it merits the distinction of having first demonstrated that something more reproducible than guesswork could be applied to these problems. The researches thus instigated paid rich dividends during World War II when the armed forces were faced with serious losses from the deterioration of all types of material.

One of his greatest contributions to applied microbiology was made during his years as Chief of the Division of Soil Microbiology, namely the development of a practical means of controlling the so-called Texas Root Rot of cotton, caused by *Phymatotrichum omnivorum*. In characteristic fashion, upon assuming this position Thom immediately familiarized himself with the vast literature upon the microorganisms of the soil, their interrelationships, and what was then known concerning the useful application of these to agronomic practices. For many years the cotton root rot had been a scourge in much of the best cotton growing regions of the Southwestern States. By careful observation and analyses he and his associates, notably Marie B. Morrow and Francis E. Clark, determined that *Phymatotrichum* could not thrive in environments characterized by a diverse and active microflora. First, they

discovered that the fungus could be "contained" if readily decomposable plant materials were added to the soil as amendments during the growing season, and subsequently it was discovered that the pathogen could be controlled if the cotton plants were plowed under immediately after harvest, thus in effect rotting out the rot before it overwintered. This is now standard agronomic practice throughout the Southwest and *Phymatotrichum* is no longer a limiting factor in the areas where it formerly was rampant.

Dr. Thom made many direct and important contributions to mycology and to microbiology in its broadest sense. His indirect contributions to these fields were perhaps equally significant. He gained much from his contacts with outstanding biologists, and as he became an established scientist, he was always willing to give of his time and counsel to everyone who sought his opinions and advice, and they were legion. Working in Washington at the crossroads of a nation's scientific effort, a steady stream of investigators with diverse interests and problems passed through his laboratory. Many of these were the "greats" of that period, and many were young scientists just starting their careers. To each he gave unstintingly of his time, and in the ensuing conversations the course of untold research investigations were influenced and oftentimes redirected. Although direct and outspoken in his writings and sometimes almost brusque in his speech, Dr. Thom loved people and by his own admission preferred talking with a fellow scientist more than the theater, the movies, the concert hall or the day's best-seller. During his active career in the Department of Agriculture Thom identified thousands of cultures for other investigators all over the world, taking little cognizance of the credit that he might or might not receive. He was withal a selfless and devoted public servant.

Dr. Thom was most considerate of those who worked for and with him. This is not to say that he encouraged laxity or procrastination; quite the opposite, for having learned early to discipline himself he naturally expected the same conduct of his colleagues. He had worked hard for his education and for his place of eminence in his profession and he quite naturally expected others to do the same, otherwise they should have chosen some other field of work. In dealing with his professional staff he was scrupulously careful not to take advantage of his position as Chief. He practiced as he believed that every man should carry his own share of the load, and, having done this, he should receive due recognition for his professional knowledge and for his contributions to the solution of the problems at hand. Commenting upon

one tendency which seems to be growing in some research organizations, he had this to say:

> "I wrote my own manuscript. The ghost writer is deemed essential to the literary reputation of some types of public men. His existence in American political fields has become so well known that his exploiters are beginning to get the contempt they deserve. In the scientific service, he is also present but superfluous. Those who live on the job with a man are not received. I long ago established the rule that I would sign nothing which I could not write. It is easy to write a letter of transmittal to carry forward what your assistants produce. To me, it is essential to self-respect."

He was most anxious that the younger men in his employ have every possible opportunity to improve their position by additional education, and often at considerable inconvenience to himself and his researches, as in the writer's case, he arranged for them to take leaves of absence that they might work for higher degrees. They were urged to take advanced courses in the night classes offered in the universities of Washington and in the Graduate School of the Department of Agriculture which he had helped to organize and in which he offered courses from time to time. He firmly believed his father's dictum: "Without an adequate education a man is soon pumping a dry well."

Dr. Thom belonged to many professional societies, and in the affairs of most of these he took an active part, for he strongly believed that a man owed it to his profession to support its journals and to do everything possible to promote the active interchange of ideas among its advocates. Only a few of these associations will be cited. He was a charter member of the Mycological Society of America and its president in 1953. He was for nearly 50 years a member of the Society of American Bacteriologists and its president in 1940. He was the temporary chairman of the organizing committee and the first president of the Society for Industrial Microbiology. He was at one time president of the Botanical Society of Washington and also of the Washington Academy of Sciences. He was a charter member of the American Dairy Science Association and died in May before its Golden Anniversary Meeting in June, at which he had been invited to speak. He was a member of the National Academy of Sciences for nearly two decades, having been elected in 1937. One of the most appreciated honors that came to him was an invitation for his wife and him to visit Spain in 1947, where he was elected an honorary member of the Consejo Su-

perios de Investigaciones Cientificas and presented with a gold medal in recognition of his outstanding services to microbiology and medicine.

During his *un*retiring retirement after 1942, Dr. Thom acted as consultant to a number of industrial laboratories, and was a frequent guest speaker on university campuses and at local and national meetings of many societies and trade associations. The breadth of his knowledge and experience, the picturesqueness of his speech and his wide acquaintance with fellow scientists—none of whom were either idolized or feared—made him a popular lecturer. Such lectures were always entertaining—but to amuse his audience was never their primary purpose, for woven into their very fiber were basic and obvious truths always presented with pointed relevance to the topic being discussed. The spirit of these can be surmised in the addresses and special lectures which are listed among his published papers. Dr. Thom was a biologist first and foremost, and he deferred to no other profession in its contributions to man's progress.

Dr. Thom was an ardent prohibitionist, a conservative in politics, and a deeply religious man. Like his forebears, he was a staunch Presbyterian and wherever he lived took a very active part in the activities of the local church. Because it reflects the sincerity of this other facet of his life and, more particularly, because it reveals the philosophy by which he lived, the following *credo* found in his papers is quoted. The page is entitled, "A personal prayer of thanks toward the evening of life":

> "Father in Heaven, I thank Thee for the opportunities of a long life; for parents who gave of themselves that I might be better prepared to live and serve; for teachers who showed the way to usefulness; for health and strength that made it possible to do the day's work uninterrupted for many years; for a clear smoothly working mind equal to the demands of life; for friends who lent help when help was needed; for work that called for daily attention; for the fortitude that never faltered though at times the vision was not clear; for faith that found joy in the work of today and confidence to prepare for tomorrow; for chances to help men upward toward higher ideals and better living; for home, and wife and children and the privilege of serving; for state, and community relations; for a measure of thy spirit that has sustained me even in mistakes and error; for the ability to leave the future to the promise—'Where I am there ye will be also.'
>
> "So let me go on each day in service, confident that for this day

my place is here, and that when Thou shall call me elsewhere Thou wilt substitute Thy strength for mine.

"Let me rejoice then in what Thou hast given, and may those I leave behind forget to weep in the joy that Thou has given me so much to share with them while here.—Amen."

Charles Thom lived a very full and rewarding life. His health remained exceptionally good until a few months before his death, and he lived to see the fulfillment of many dreams which provided deep satisfaction for his latter years. At his request, he was buried in Storrs, Connecticut, a community which he never ceased to regard as "home." It was there that he began his professional career; it was there that he built his first house and there that his children were born. He is survived by his son, Charles Richard, an attorney in Port Jefferson, New York, and two grand-daughters.

DEPARTMENTS OF BACTERIOLOGY AND BOTANY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

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EDWARD M. GILBERT 1875–1956

M. P. Backus and H. C. Greene

(with portrait)

Emeritus Professor E. M. Gilbert of the University of Wisconsin died at his winter home, San Marcos, Texas, on April 23, 1956, in his eighty-first year. In his passing, American science lost a man who had been a prominent and colorful figure in its mycological, phytopathological, and botanical circles since the second decade of the century. For nearly twenty years chairman of the Botany Department at the University of Wisconsin and for more than thirty years mycologist at that institution, Dr. Gilbert helped train scores of plant scientists who today are active workers throughout the United States and in several foreign lands. Although the list of his own mycological publications is not long, Dr. Gilbert had a strong interest in mycological research; he initiated and supervised many investigations dealing with the fungi. A very active member of the Wisconsin Academy of Sciences, Arts and Letters and of several national scientific organizations, he exerted, through these channels, a substantial influence on the scientific life of his state and country. He was also co-author of two widely-used botanical textbooks.

Edward Martinius Gilbert was born September 20, 1875, at Blair, Wisconsin, the eldest son of Thomas I. Gilbert, a pioneer merchant, and of Julia Jahr Gilbert. He was of Scandinavian ancestry, his father having been born in Valdres and his mother in Enebak, Norway. In 1910 he was married to Esther Montgomery Lowry who, with two sons, a daughter, and seven grandchildren, now survives him.

Although mycology was to become Dr. Gilbert's leading scientific interest, he did not enter this field of specialization until he was past thirty years of age. He began his teaching career in 1901 at the Hayward High School in northern Wisconsin, serving also as principal of that school. In 1907 he received his Ph.B. degree at the University of Wisconsin, where he came under the influence of Professor R. A. Harper, an inspiring teacher and an investigator who at that date had already won international renown for his pioneer work in the field of fungal cytology. This contact with Harper gave direction to Dr. Gilbert's future career. After an interval of three years, during which he

EDWARD MARTINIUS GILBERT
1875–1956

served as professor of biology at the State Normal School in Superior, Wisconsin, he returned to the University of Wisconsin for graduate work and completed a mycological thesis under the joint supervision of Professor Harper and Professor C. E. Allen. He was awarded the Ph.D. degree in 1914. Dr. Gilbert was made an assistant professor in the Botany Department at the University of Wisconsin in 1913 and, with the departure of Professor Harper, who had accepted a post at Columbia University, he took over the instructional work in mycology. During 1915, on leave, he worked with Professor Roland Thaxter at Harvard. Soon afterwards he was advanced to an associate professorship at Wisconsin, and in 1922 was appointed Professor of Botany and Plant Pathology, a position which he held until his retirement in 1946.

Fungal cytology was undoubtedly the area of Dr. Gilbert's greatest competence. However, his mycological interests and activities were by no means confined to this field. He was, for example, also much interested in the taxonomy of the jelly fungi, of *Clavaria,* and of various phycomycetous groups. From an early date, beginning indeed with the period when he worked in Dr. Thaxter's laboratory, Dr. Gilbert was attracted to the entomogenous fungi. At one point in his career he was for a short time able to concentrate all his attentions on this mycological area, for in 1925 the Florida Agricultural Experiment Station and the Plant Board of Florida engaged him to study the fungal diseases of a destructive citrus aphid, a program in which he was associated with Dr. W. A. Kuntz. Plant pathogenic fungi of all kinds also interested Dr. Gilbert. His enthusiasm in connection with these forms was fanned by his official and strong unofficial ties with the Plant Pathology Department of his University, as well as by the predilections of his students, many of whom were majors or minors in plant pathology. His attention to this field was further greatly stimulated by his close associations with Dr. J. J. Davis, who had come to the Madison campus in 1911 to become curator of the Cryptogamic Herbarium and who in the subsequent quarter of a century conducted an exceedingly thorough study of the parasitic fungal flora of Wisconsin.

A confirmed early riser, Dr. Gilbert was usually at work before seven o'clock in the morning, and at this hour of the day he could most often be found preparing laboratory materials for his classes or filling the blackboard with the elaborate sketches of fungi which he used so effectively in his teaching. The colorful classroom personality of this man made a lasting impression on all who studied under him. He had a rather high-pitched and distinctive voice, and a large store of anecdotes,

not malicious, but in some cases highly entertaining, about prominent mycologists and pathologists. With characteristic enthusiasm he would interlard his lectures at appropriate points with these stories, and the effect was to give the student intriguing behind-the-scenes glimpses at the development of mycological science in this country. The personal interest which he took in his advanced students was remarkable. Years after they had left the campus he could still recall clearly all the members of a given class; furthermore, he knew where most of them were located and what they were doing.

Dr. Gilbert devoted much time and energy to the advancement of projects falling outside the realm of his scientific specialties. He was an ardent conservationist who worked enthusiastically for the preservation or restoration of natural areas in his state. For fifteen years he gave freely of his energies in the task of founding and developing the Arboretum of the University of Wisconsin, and his love for the wild country undoubtedly was influential in shaping the unique policy of this arboretum to include the establishment of a series of native forest and prairie habitats each as complete in representation as possible. In another direction, it was largely through his urging and guidance that the Wisconsin Junior Academy of Science was founded in 1944. This organization has been of increasing importance in the encouragement of science-talented young people of Wisconsin.

Dr. Gilbert belonged to many scientific organizations, including the Mycological Society of America, the British Mycological Society, the American Phytopathological Society, the Botanical Society of America, the American Microscopical Society, the American Society of Naturalists, the American Society of Taxonomists, and the Wisconsin Academy of Sciences, Arts and Letters. He was a fellow in the American Association for the Advancement of Science. After his retirement and when he and Mrs. Gilbert had taken up winter residence in the South, he joined the Texas Academy of Science.

Near the end of his teaching career Dr. Gilbert developed a heart ailment which forced him to curtail his physical activities. However, until his final illness struck, he was never seriously incapacitated and he thoroughly enjoyed his ten years of retirement, during which he was able to spend long summers at the family cottage in the Wisconsin northwoods, close to his favorite fungal collecting grounds. His pattern of living in retirement included a modest program of mycological and pathological work and keeping abreast of developments in other botanical fields.

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NOTES AND BRIEF ARTICLES

Two new phalloids from Taiwan

The two species here described attack the underground parts of *Dendrocalamus latiflorus* Munro, causing rot.

Dictyophora cinnabarina sp. nov.

Solitaria, parasitica; pileo conico, cinnabarino, reticulato; materiam sporiferam foetidissimam obtegente; apice annulato; stipite terete, lacunoso-spongioso, elastico, cavo, superiore cinnabarinis; volva sordide alba vel brunnescente; sporis irregularibus, plurimis obovatis-ellopsoideis vel subclavatis, 3–4 × 1.6–2.2 μ.

Expanded fructification consisting of a gelatinous volva, an elongated stipe, a pileus and an indusium; pileus conical, with ring at apex, bearing the mucilaginous, dark-brown, fetid gleba, which, when this has been removed by flies, reveals the reticulate, cinnabar-red surface; indusium attached at apex of stipe under the cap, free from the pileus, pendent 2–10 cm below lower margin of cap, netted, the meshes smaller nearer the margin, brilliant cinnabar-red, or paler, but becoming brilliant red when exposed to strong sunlight; stipe hollow, 7–13 cm tall, 0.8–2 cm in diameter, tapering upward or nearly cylindrical to near the apex where it is narrowed to the blunt, perforated tip, cinnabar-red above, fading to paler below; spores irregular, mostly obovate-ellipsoid or subclavate, brownish green, 3–4 × 1.6–2.2 μ.

Unexpanded fructification ovate, rather small, 2.5–5 × 1.8–3.5 cm, grayish white or dingy brown, with 1–2 long, well-developed, pinkish rhizomorphs at base; gelatinous-pliant due to the gelatinous inner layer, rupturing by an irregular slit.

Taiwan (Formosa): Scattered on wet soil in shade of *Dendrocalamus latiflorus* Munro, on the banks of the Tam-Sui River near Taipei, August 28, 1945.

The fructifications appear singly, never gregariously, in late August, during very wet seasons only. Under favorable temperature conditions, the stalk may become fully expanded in half an hour after the peridium of the egg splits.

The flies which favor this species are the green bottle flies of the genus *Lucida,* not the house-flies, *Musca,* which swarm upon *D. phalloidea* Desv., *D. indusiata* (Pers.) Fisch. and *D. duplicata* (Bosc ex Fr.) Fisch. The dried stipes and indusia of *D. phalloidea* are used for

a highly esteemed Chinese dish. Should those of the present species be inadvertently used in preparing such a dish, the flavor would be markedly affected because of the particularly offensive odor of *D. cinnabarina*.

Phallus formosanus sp. nov.

Solitarius, parasiticus; pileo tenue, conico, rubro, granuloso; materiam sporiferam brunneo-atrem, foetidissimam abtegente; apice annulato, rubro; velo nullo; stipite terete, elongato, cavo, vagina cylindracea circumvalente, 10–14 tubularum

FIG. 1. *Dictyophora cinnabarina,* egg and mature basidiocarp, × ½.

coalescentium composita; volva alba vel punica; sporis ellipsoideis, levibus, 2.7–3.8 × 1.4–2 μ.

Expanded fructification consisting of a gelatinous volva, an elongated stipe and a pileus; pileus conical, with perforated red ring at apex, bearing the blackish brown, fetid gleba, the exposed surface granular, not reticulate, deep red, thin, consistently rupturing at the margin by slits due to the expansion of the pileus before elongation of the stipe; stipe hollow, red, 10–15 cm tall, 0.9–2 cm in diameter at the swollen lower portion, narrowed upward, encircled for half its length by a red cylindrical sheath composed of 10–14 united tubes, closed at both ends, the sheath homogeneous with the stipe and united to it except at the upper and lower ends; spores ellipsoid, smooth, greenish yellow, 2.7–3.8 × 1.4–2 μ.

Unexpanded fructification smooth, ovate, 2–3.5 × 1.4–2.6 cm, white or pinkish, gelatinous-pliant, with a short, white rhizomorph at base.

Taiwan (Formosa): Scattered on wet soil in shade of *Dendrocalamus latiflorus* Munro, on the banks of the Tam-Sui River near Taipei, December, 1945 and January, 1946.

The fungus fruits singly on the ground under the bamboos in winter, chiefly in December and January. On the basis of clinical experiments covering several years, it produces a substance that acts effectively on

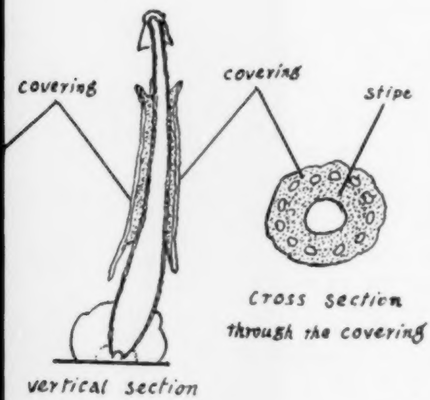

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FIG. 2. *Phallus formosanus*, mature basidiocarp, showing sheath encircling stem and characteristic splitting of pileus, × ½.

FIG. 3. *Phallus formosanus*, diagrammatic longitudinal and cross sections showing relation between stem and sheath.

leucaemia. Whether or not it acts directly on the pathogen (virus of Dr. L. Gross) must be determined by future research.

The original specimens have been ruined and the type locality has been destroyed, partly by floods, partly by building operations. Under the circumstances, it seems permissible to publish descriptions of these species.—WEI SIANG LEE, No. 7, Lane 17, 1st Sect., East Chung-Ang Road, Taipei, Formosa, China.

EAR FUNGI

During World War II and in the years following, an unprecedented number of people from temperate climates had to live in tropical countries where the uniformly warm temperature and high relative humidity encourage the growth of fungi, particularly pathogenic ones. As a result of the dramatic increase in the number of skin infections, an increased awareness of fungi as human parasites could be observed. In fact, the idea that fungi cause dermatitis became so widely accepted that other sources of difficulty were frequently overlooked. Such a notion was particularly common when applied to ear infections, which most sufferers from otitis referred to popularly as "fungus in the ears" regardless of the actual cause of the malady.

In order to determine to what extent fungi are involved in human otitis, smears were taken, before any treatment was started, from fifty cases which reported to U. S. Naval Dispensary, Coco Solo, Panama, Canal Zone. To encourage growth of any fungi which might be present, streaks were made on glucose-yeast-extract agar (0.5% glucose, 0.1% Difco yeast extract, salts as in Czapek's). Identifications were made later from colonies on Czapek's agar.

In every one of the fifty cases encountered, bacteria were present and apparently involved. Thirty contained *Staphylococcus aureus,* ten *Pseudomonas* sp., and ten unidentified gram-negative rods.

Fungi were cultured from only ten of the cases, and these were common *Aspergilli* which could well have been air contaminants whose spores were simply lying in the ear canal. Six cases produced *A. flavus,* a species which has been implicated in ear infections many times, but which has not been proved as a pathogen. Members of the *A. niger* series, which has a history similar to that of *A. flavus,* appeared three times. One case provided a colony of *A. flavipes,* a species which has not been previously reported from human ears, but which is known for its ability to thrive under conditions of relatively low humidity.

An observation of interest is the remarkable lack of cerumen in the affected canals. This lack, noted in every case in the present series, may be either a predisposing cause or an effect of the infection.

Several facts make it unlikely that the isolated fungi caused otitis. The treatment consisted of daily and thorough cleaning of the ear canal, insertion of Burow's wicks (containing lead acetate), and insufflation with sulfadiazine powder, or application of cresatin, terramycin, bacitracin, or other antibiotics. Among fungi, only a non-pathogen or a weak secondary invader would be likely to succumb to these measures.

Further, the isolation of bacteria of known pathogenicity from most of the cases absolves the molds from some of the direct implication. Such fungi as were isolated were of doubtful pathogenicity. Finally, the relatively low number of cases which did involve fungi makes the conclusion apparent that what is generally called "ear fungus" in the tropics is more probably something else. DON RITCHIE, Dept. of Botany, Barnard College, Columbia University, New York 27, and J. J. ZARRIELLO, Cdr. (MC), U.S.N., U. S. Naval Station, Coco Solo, Canal Zone.

A NOTE ON THE OCCURRENCE OF CERTAIN AQUATIC FUNGI IN FLORIDA [1]

The writers wish to call to the attention of fellow mycologists the year-round occurrence in north Florida of such fungi as *Rhipidium, Blastocladia, Mindeniella, Monoblepharis,* and *Gonopodya.* The records of their presence in this state may or may not be new, but the ease with which they can be found, even during the hot summer months, is most unusual.

Many sinks are to be found in the Tallahassee region of north Florida. These have been formed by the dissolution of the porous limestone near the surface and the subsequent caving in of the overlying sands. Many of the lakes of this region and central Florida occur in large and deep sinks, the bottoms of which extend below permanent ground water level and are not a perched condition from land drainage. Many of the sinks are quite large. One, known locally as "Dismal Sink," is about 250 feet in diameter at the rim and 60 feet deep to the water level. Others are much smaller. Water is usually present in the sinks, and it is believed that many may be connected by underground streams.

The temperature of the water in these sinks varies only about 5 degrees during the year, with an average of about 21° C. Even during the hot summer months, water temperature is seldom over 22° C, while that of the open cypress swamps feels like a warm bath to the hand. Wakulla Springs, near Tallahassee, varies only between 20° and 21° C during the entire year. Temperatures recorded at the time of investigation verify the above statement.

This phenomenon, therefore, affords the mycologist an opportunity for collecting the forms mentioned below even during the hot summer months. Recently, and in previous years, the authors have collected in

[1] Contribution No. 69, Botanical Laboratory, Florida State University.

sinks and Wakulla Springs the following forms, using apples and twigs as bait, during every month of the year:

1. *Blastocladia pringsheimii* Reinsch.
2. *Blastocladia* sp. This form has not yet been identified. The colonies on apples are bright lemon yellow and orange in color.
3. *Rhipidium americanum* Thaxter.
4. *Mindeniella spinospora* Kanouse.
5. *Mindeniella.* Unidentified. Found growing on apple.
6. *Gonopodya polymorpha* Thaxter.
7. *Gonopodya prolifera* (Cornu) Fischer.
8. *Monoblepharis polymorpha* Cornu.

These fungi, then, are readily available for study during the entire year. The authors have made no serious study of the taxonomy of these fungi but it is hoped that this may be done in the near future.—A. W. ZIEGLER AND BETTY LINTHICUM, Florida State University, Tallahassee, Florida.

SUGGESTIONS FOR CONTRIBUTORS TO MYCOLOGIA

At the present time, each volume of MYCOLOGIA contains approximately 900 pages. This is as much as funds available permit us to print. The rising cost of printing will in all probability make it necessary to reduce this number of pages unless increase in membership and in dues keeps pace with the cost. Even now, it is necessary to reject worthy papers and to return others with the request that they be shortened substantially.

In the limited space available on the inside back cover it is impossible to print directions for preparation of manuscript which will care for all possible contingencies, but too many contributors fail to consider what is given there. One of these is the matter of double-spacing. It is often assumed that this applies only to the body of the text and that foot-notes, tabular data, literature citations, author's address, explanation of figures, etc. may be single-spaced. Such items are usually those which require the greatest amount of editorial marking between the lines. EVERYTHING should be double-spaced.

There are few papers which, as submitted, could not be shortened to some extent without omitting anything of importance. Many could be very substantially shortened. This is admittedly a difficult problem for an editor, since only a specialist in any field can decide what is essential

and what may be omitted. But clumsy and circumlocutory phraseology can often be replaced by simple, direct statements, resulting not only in shortening the text, but in improving the presentation.

Papers involving experimental work usually require tables, but tabular material is very expensive to set up and should be kept to the minimum essential to substantiate results. Lines are sometimes necessary, particularly in complicated tables, but in many cases they are not essential and in such cases they should be omitted. Excessive duplication, in the text, of data given fully in tables or graphs may often be omitted with definite gain to clarity of presentation.

Taxonomic papers, by their nature, take a great deal of space, and when new taxa are proposed, Latin diagnoses are necessary. It should be pointed out, however, that the Latin diagnosis need not be a literal translation of the full English description. It should be shortened to give the essential facts and the necessary elaboration may be left to the vernacular discussion. A very complete Latin description, omitting the English diagnosis, is equally satisfactory, but will probably meet with less favor. In no case is it necessary to repeat collection data given in one language by a translation into the other, and such duplication will always be deleted.

New taxa should always go to the left margin, where they will be printed in bold-face type, and the validating Latin diagnosis should follow immediately as a complete paragraph. Such indications as "sp. nov." etc. should appear *only* in connection with the valid publication; especially not in the title. This is a matter often disregarded, and is a frequent source of confusion in citations.

Bibliographies are always printed in alphabetical order. References to them in the text may be either by date (Jones, 1953), or by number (6). If there are two or more authors, the initials of the first author only are printed after the surname, e.g., Smith, J. L. and B. B. Brown, NOT Smith, J. L. and Brown, B. B.

The title of a journal or other publication cited should be given, with appropriate abbreviation, for every reference. "Ibid.," "loc. cit." etc. may be clear or they may be extremely confusing. Because it is impossible to draw a line between the two extremes, it is not desirable to use them.

In giving microscopical measurements, it is frequently desirable to indicate exceptional dimensions within parentheses. When this is done, it should be in the following form: (10–)12–15(–16.5) × (4–)5–6 μ, NOT (10–) 12–15 (–16.5) nor (10)–12–15–(16.5). Most typewriters do not have the letter μ; in such cases, a lower case u with a *straight*

pen line at the left side makes a good substitute. These are little things, but their neglect makes extra work for the editor.

Authors sometimes cite microscopic dimensions in hundredths of a micron. A figure for spore size given as $11.79–13.21 \times 4.84–5.17\ \mu$ (such have been received) leads to speculation, not only as to the methods used to arrive at such precision, but also concerning the mental processes of the author.

Legends for illustrations are frequently pasted on the bottom of the figures. As the illustrations go to the engraver and the legends to the printer, this means that the editor must retype the legends or hold the paper until the author has done so. Space should always be provided at the bottom of the figures for editorial directions to the engraver. The amount of reduction desired should be indicated and magnifications given in the legends should apply to the figures as reduced.

Over a period of many years MYCOLOGIA has established a format. This is not fixed, but has developed and is developing. No one would contend that it is the best possible format, but few would claim that any paper should be printed in any form any author should choose to adopt. Furthermore, there is a good deal of work involved in preparing papers for publication and this is very greatly lessened by reasonable standards of uniformity. There is, therefore, a very real obligation imposed on those who submit papers to MYCOLOGIA to consult comparable papers in recent issues and to arrange their papers in accordance with current usage of the journal. If the usage ought to be changed, suggestions to the Board of Editors will always receive careful consideration.

Much of what is stated here was published in the December, 1955, News-Letter. Apparently many members of the Society failed to read it. It is hoped that by printing it in MYCOLOGIA it will reach most of our future contributors.—G. W. MARTIN.

Report of the Managing Editor of Mycologia

Statement of cash receipts and disbursements for the year ended June 30, 1955

Cash receipts		
Joint funds		
Members' subscriptions (Mycological Society)	$ 3,167.50	
Other subscriptions	6,172.98	
Sales of V. 25 and later	608.56	
Excess pages and illustrations	724.43	$10,673.47
Special funds		
Sale of V. 1–24 and Index	605.50	
Income from endowment	725.00	1,330.50
Total receipts		$12,003.97
Cash disbursements		
Printing and distribution	11,394.00	
Reprinting	440.83	
Office Expense	319.02	
Total expenditures		$12,153.85
Excess of expenditures over receipts		149.88
Unexpended balance July 1, 1954		4,295.97
Unexpended balance July 1, 1955		$ 4,146.09
Endowment		$14,000.00

The above Mycologia funds are administered by The New York Botanical Garden, and the balances at June 30, 1955, are in agreement with the amounts shown in the financial statements of that organization which have been examined by Price, Waterhouse & Company.

Donald P. Rogers, *Managing Editor*

REVIEWS

MILDIOUS, OIDIUMS, CARIES, CHARBONS, ROUILLES DES PLANTES DE FRANCE, by G. Viennot-Bourgin. Vol. 1, 317 pp. Vol. 2, 98 pl. Quarto. Paul Lechevalier, rue de Tournon, Parie, VI^e. 1956. Price 18,000 frs. (about $52.).

These volumes constitute numbers XXVI and XXVII in the series issued by the firm of Paul Lechevalier under the general title of Encylopédie Mycologique. The author, well known in the world of mycology and plant pathology, brings together in readily usable form his knowledge gained through 30 years of teaching and research of the rusts (Uredinales), smuts (Ustilaginales), powdery mildews (Erysiphaceae) and downy mildews (Peronosporales) occurring as plant parasites in France. The host genera, comprising both native and cultivated plants, are presented alphabetically as major headings, each with a numbered list of the species involved. Under each such heading the fungi of the four groups listed above known for France are then given with a brief diagnosis of each and reference to host species by number. Illustrations occurring in volume 2 are cited. A glossary of technical terms and an index are included. A very considerable number of the fungi treated in the text are superbly illustrated in the plates making up volume 2. Features included are a habitat sketch of each fungus as it appears on its host and microscopic details of spores and conidia, their mode of germination, asci, conidiophores and other morphological details.—J. A. STEVENSON.

PRINCIPLES OF FUNGICIDAL ACTION, by James G. Horsfall. ("A New Series of Plant Science Books," Vol. 30), pp. i–xx, 1–280; figs. 1–16, tables 1–11. The Chronica Botanica Co., Waltham, Mass., and Hafner Publishing Co., New York City. 1956. Price $6.50.

The average mycologist will have crossed unchartered territory and seen new horizons for future studies on the fungi after reading this volume. If ever a book mirrors a man, this one does the author. His personality is reflected in the realism of phrasing, the unhedged freshness of thought and the concepts that are the frontiers of the science—perhaps even at times beyond. This is no second edition rehash of his earlier work "Fungicides and their Action" nor a compilation of how and what fungicides to squirt or dust, but a readable exposition of the mechanism of toxicity and how certain types of compounds may bring

it about. It is well documented, although unashamedly weighted with data and ideas developed by the Connecticut Agricultural Experiment Station's staff—past and present. Some of the ideas expressed are certain to stimulate arguments by the readers—and perhaps stimulate their thinking. A great deal of the book must be savored mentally to extract the greatest information and enjoyment for the research-minded.

The colloquial style will irritate some and please many readers. Perhaps economy prompted the publisher to interlard the "Table of Contents" with several historical classics on alternating pages, but the introductory chapter might have been better placement. The first chapter starts the "fight with the fungi" when "man came down from the trees" and in a few pages chronologically cites the men and agents that made significant advances in the battle. The fungicide concept is defined and the killing action of fungicides limited to the terms protection or therapy. Chapters II and III discuss the measurement of fungicidal action and protection, followed by chapters on the "mobilization" of a protectant fungicide and how it may permeate into a fungus.

Next the discussion turns to the disruptive effect or physical toxicity of chemical toxicants on internal cellular structure and then to mitotic and morphological aberrations. Two chapters are devoted to the "metabolic squeaks" that "convert symphony to cacophony" in the life processes of the fungus itself and to the importance of metal chelation. The vocabulary is such that the biochemist will shake his head vertically, while the taxonomist may only nod. The action of metals, elemental and organic sulfur, quinones, and heterocyclic compounds on the metabolism of the fungus are discussed on 72 pages largely to link molecular structure with biological activity.

As a leader in the now popular field of plant chemotherapy, the author uses the final and fifteenth chapter to express his views and summarize the advances to date. We learn why all chemotherapeutants are not systemic fungicides. The possible mechanisms of chemotherapeutic activity for bacterial, fungous, and viral infections are discussed, but they hold more promise than practical application to disease control at the present.

"Environing factors," "elementary sulfur," and "systematic insecticide" are phrases which will cause some to grab their dictionary, but these are hairs on the elephant's tail compared to the mass of sound sense given us in the book. The "meat" is summarized at the end of most chapters for habitual abstract readers. A bibliography of 27 pages validates the author's statements and provides pay dirt for the fungicide prospector.—F. L. HOWARD.

MANUSCRIPT

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